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FAB 17

MICROBIAL TOXINS IN FOODS

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FOOD SCIENCE AND TECHNOLOGY ABSTRACTS

under the direction of

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INTRODUCTION

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Titles of the FABs now available are given on the back cover of this booklet. New titles are being added at the rate of about 10 per year. For up-to-date lists of FABs or suggestions for new topics please write to the address given overleaf. New subjects are searched for at least the five most recent volumes of Food Science and Technology Abstracts. Thereafter each FAB is updated monthly. Copies of each month's abstracts on any topic may be obtained as indicated on the back cover of this publication. At the end of each volume of up-dating, the abstracts are merged and made available as a separate supplement to the original FAB.

Some of the larger FABs have been divided into sections to facilitate use. Abstracts are not printed in more than one section. The larger FABs also have subject indexes provided.

Copies of all original articles referred to in the abstracts may be bought (or occasionally borrowed) from the International Food Information Service. A form for ordering these is provided at the end of this FAB.

Coverage of the subject has been restricted to that of Food Science and Technology Abstracts, which covers over 1200 of the important food journals, patents from 20 countries and books published world-wide. Every effort is made to include all significant references, but editorial discretion is used on the many articles of borderline interest. If the reader particularly needs an exhaustive search of the subject, we will be pleased to provide any other references that we have available. We would, in any case, encourage readers to write or telephone us with any comments or queries that they may have.

H. BROOKES

ASSISTANT EDITOR

1

Use of aggregate-hemagglutination technique for determining exo-enterotoxin of *Bacillus cereus*.

Gorina, L. G.; Fluor, F. S.; Olovnikov, A. M.; Ezepeuk, Yu. V.

Applied Microbiology 29 (2) 201-204 (1975) [21 ref. En] [Gamaleja Inst. for Epidemiology & Microbiol., USSR]

The possibility of using the aggregate-haemagglutination technique for detection of *B. cereus* exo-enterotoxin in foodstuffs and culture media is shown. 0.004 µg enterotoxin/ml can be detected by this method. AS

2

Reassessment of the coagulase and thermostable nuclease tests as means of identifying***Staphylococcus aureus*.**

Rayman, M. K.; Park, C. E.; Philpott, J.; Todd, E. C. D.

Applied Microbiology 29 (4) 451-454 (1975) [14 ref. En] [Health Protection Branch, Health & Welfare Canada, Ottawa K1A 0L2, Canada]

A total of 91 enterotoxigenic strains of *Staph. aureus* isolated from foods and tested for production of coagulase and thermostable nuclease and the ability to ferment glucose and mannitol anaerobically, and glucose aerobically showed, with the exception of 4 strains, a complete correlation among these properties. A similar correlation was observed with 103 cultures of *Staph. aureus* isolated from clinical material. In all instances, the coagulase reactions were sufficiently strong to be scored at either the 3+ or 4+ levels. Presumptive staphylococcal cultures isolated during routine examination of foods and yielding 2+ coagulase reactions or lower were invariably negative for thermostable nuclease production. It is suggested that the thermostable nuclease test be performed on cultures with doubtful coagulase reactions before classifying them as *Staph. aureus*. AS

3

A new CoDNA-ase spot plate method for the detection of coagulase and DNA-ase activity of staphylococci.

Mol, H.; Vincentie, H. M.

Tijdschrift voor Diergeneeskunde 100 (8) 426-431 (1975) [33 ref. En, nl] [Lab. of the Food Inspection Service, Utrecht, Netherlands]

The test organisms are plated on CoDNA agar medium (Oxoid DNA-ase agar containing Bacto coagulase plasma or diluted swine plasma) and incubated for 18-24 h at 37°C. Coagulase-positive colonies are black and surrounded by a zone of precipitation on a clear plate; after flooding the plate with 1N HCl, DNA-ase activity is demonstrated by a clear zone around the precipitation zone on a hazy clouded plate. In tests on 125 human and 226 bovine (mainly mastitis)

strains, and 340 suspect strains and atypical strains from food isolated on Baird-Parker's medium, good agreement was found between the CoDNA-ase method and conventional tests for coagulase and DNA-ase activity. The 2 forms of plasma gave similar results in the conventional coagulase test; swine plasma was the cheaper, and despite certain difficulties that might be encountered in interpreting results on CoDNA plates, this form of plasma is recommended for use in routine tests by the CoDNA-ase method. CDP

4

[Staphylococci in foods - a health hazard?]

Staphylokokken in Lebensmitteln - ein gesundheitsgefährdendes Problem für den Konsumenten?

Bendig, J.

Ernährungsforschung 20 (1) 22-25 (1975) [De] [Zentralinst. für Ernährung, Potsdam-Rehbrücke, German Democratic Republic]

The problem of food-poisoning attributable to staphylococcal enterotoxins is discussed, with special reference to its significance for catering institutions. Aspects considered include: factors influencing growth and toxin formation by staphylococci in foods; sources of contamination; foods commonly implicated in staphylococcal food poisoning; and hygiene measures to minimize the danger of staphylococcal food poisoning. IN

5

Interpretation of the tube coagulase test for Identification of *Staphylococcus aureus*.

Sperber, W. H.; Tatini, S. R.

Applied Microbiology 29 (4) 502-505 (1975) [15 ref. En] [Pillsbury Co. Res. & Development Lab., Minneapolis, Minnesota 55414, USA]

The tube coagulase test is a valid means of identifying *Staph. aureus*, provided that only a firm clot that does not move when the tube is tipped is considered a positive reaction. The widely promulgated interpretation that all degrees of clotting in coagulase plasma are a positive identification of *Staph. aureus* was disproved by the use of other tests such as anaerobic glucose fermentation, thermonuclease production, and lysostaphin sensitivity. Source of supply of the coagulase plasma was a factor in the occurrence of false-positive coagulase test results. The use of a mixture of pig and rabbit plasma in the tube coagulase test is also discussed. AS

6

Deoxyribonucleic acid base composition and biochemical properties of certain coagulase-negative enterotoxigenic cocci.

Lotter, L. P.; Genigeorgis, C. A.

Applied Microbiology 29 (2) 152-158 (1975) [37 ref. En] [Inst. für Lebensmittelhygiene der Freien Univ. Berlin, 1 Berlin (West) 33]

8 coagulase-negative, enterotoxigenic strains of

cocci and one weakly coagulase-positive strain isolated from sources including cases of food poisoning were evaluated for their relationship to *Staphylococcus aureus* on the basis of DNA buoyant density and physiological studies. AS

7

Combined effect of water activity and pH on the growth of butyric anaerobes in canned pears.

Jakobsen, M.; Jensen, H. C.

Lebensmittel-Wissenschaft + Technologie 8 (4) 158-160 (1975) [14 ref. En] [Food Tech. Lab., Tech. Univ. of Denmark, 2800 Lyngby, Denmark]

For 7 strains of butyric anaerobes (*Clostridium* species) the combinations of pH and water activity (a_w) required for preventing growth in canned pears were investigated. Pronounced differences in the pH and a_w tolerance of the different strains were found. The most sugar tolerant of the organisms investigated did not grow at a_w below 0.975-0.970, even at pH 4.5. In the a_w range 0.975-0.985 none of the strains grew at pH 3.8-4.0, whereas growth was observed at pH 4.2-4.4. At pH 3.8, just one strain was able to grow, and this occurred only at $a_w \geq 0.985$. The extent of inhibition obtained by decreasing a_w was independent of whether glucose, fructose or sucrose was used as the a_w -controlling solute. From the results obtained the possibilities of preventing spoilage from spores surviving heat processing are discussed. The application of fp determinations for estimating a_w of brines from canned fruits is recommended. AS

8

Two outbreaks of *Bacillus cereus* food poisoning in Canada.

Todd, E.; Park, C.; Clencner, B.; Fabricius, A.; Edwards, D.; Ewan, P.

Canadian Journal of Public Health 65 (2) 109-113 (1974) [15 ref. En] [Food Res. Lab., Health Protection Branch, Health & Welfare Canada, Tunney's Pasture, Ottawa, K1A 0L2, Canada]

Preparation of chicken a la king in a Montreal hospital and of barbecued chicken in a supermarket (both implicated in outbreaks of food poisoning) are outlined. Samples of the food were analysed bacteriologically; 10 fold serial dilutions were prepared in phosphate buffers and tested for the presence of *Bacillus cereus* with spread plates of phenol-red egg yolk polymyxin B agar. Biochemical reactions and ileal loop responses in rabbits are given in tabulated form. Counts for chicken a la king and barbecued chicken were 6.0×10^4 and 6.9×10^4 cells/g, respectively. 3 strains of *B. cereus* from chicken a la king showed positive loop responses. *B. cereus* from the barbecued chicken produced a loop response index of 0.22 which is sufficient to be considered toxic. It was concluded that temp. abuse during preparation enabled *B. cereus* to grow in the chicken and cause illness.

GL

9

[Radio-immunoassay: method and application in food inspection.] Radioimmunoassay: Methodik und Einsatzmöglichkeit in der

Lebensmitteluntersuchung. [Lecture] Hebel, R.-D.

Fleischwirtschaft 55 (6) 812-813 (1975) [31 ref. De] [4150 Krefeld 1, Deutscher Ring 100, Federal Republic of Germany]

Principles and applications of radioimmunoassay (radiostereoisotopic assay) in food analysis are described. Applications include detection of hormone residues and bacterial toxins, and the study of changes in food products between production and consumption. 200 tests/wk can be carried out by 1 person (1000/wk with automation). RM

10

The influence of temperature on growth, sporulation, and heat resistance of spores of six strains of *Clostridium perfringens*.

Rey, C. R.; Walker, H. W.; Rohrbach, P. L. *Journal of Milk and Food Technology* 38 (8) 461-465 (1975) [26 ref. En] [Dep. of Food Tech., Iowa State Univ., Ames, Iowa 50010, USA]

The influence of temp. on growth, sporulation, and heat resistance of 6 strains of *Cl. perfringens* was examined. The range of temp. for growth and sporulation varied between strains and was influenced by the culture medium. Optimum growth occurred in thioglycolate medium between 30° and 40°C for all strains. Optimum sporulation in Ellner's medium occurred in the 37-40°C range for all strains. The max. number of spores produced varied with the strain. Heat resistance of the spores varied between strains but was not modified by temp. of incubation. AS

11

Spore resistance data - a base for calculating food sterilization processes? [Lecture]

Jonsson, U.

SIK Rapport No. 359, 20-37 (1974) [En] [Swedish Inst. for Food Preservation Res. (SIK), Göteborg, Sweden]

Conventional procedures for thermal sterilization process calculations are critically discussed, with reference to: the heat-inactivation process as a first-order reaction; the linear model and the Arrhenius model of dependence of the D-value on temp.; and the temp. dependence of the Z-value. Brief details are given of studies on heat-inactivation of *Bacillus stearothermophilus* at 111-125°C. Graphs of results are given, together with mathematical models relating D-values to temp. The results show that the Z-value decreases with increasing temp.; the basis of this dependence of Z value on temp. is briefly discussed. [See 8 2B18.] AJDW

12

The influence of water activity on heat resistance of bacterial spores. [Lecture]

Härnqvist, G.

SIK Rapport No. 359, 73-85 (1974) [En] [Alfa-Laval AB, Tumba, Sweden]

Studies on the effect of water activity (a_w) on the heat resistance of spores of *Bacillus subtilis* and *B. stearothermophilus* are described. To differentiate effects of a_w from specific effects of the solutes used to control a_w values, comparative studies were conducted on heat resistance in water vapour and in solutions of NaCl, LiCl, glucose or glycerol. Heat resistance of *B. stearothermophilus* spores was also determined in samples of egg powder, fish protein concentrate and wheat flour conditioned to various a_w values. Tables and graphs of results are given. The results show that changes in heat resistance with a_w are similar in water vapour and in glycerol solutions, suggesting only slight specific effects of glycerol. Heat resistance in solutions of NaCl, LiCl and glucose was lower than that in water vapour of equivalent a_w , suggesting significant specific effects of these solutes on heat resistance. Changes in heat resistance of *B. stearothermophilus* with a_w in egg powder, fish protein concentrate and flour resembled those in water vapour. The thermodynamics of heat inactivation of bacterial spores are briefly discussed. The practical significance of these studies for food preservation is considered. [See 8 2B18.] AJDW

13

[Detection of *Bacillus cereus* in foods: gas chromatographic analysis of bacterial fatty acid composition.] Nachweis von *Bacillus cereus* in Lebensmitteln: Gaschromatographische Analyse der Zusammensetzung der bakteriellen Fettsäuren. [Lecture]Niskanen, A.; Kiutamo, T.; Mälkki, Y.; Nikkilä, E. **Archiv für Lebensmittelhygiene** 26 (1) 15-16 (1975) [3 ref. De] [Tech. Res. Cent. of Finland, Food Res. Lab., SF-02150 Otaniemi, Finland]

The purpose of this work was to establish whether: (i) statistically significant differences in the fatty acid (FA) compositions of the *B. cereus* group can be found; (ii) small changes in the mass culture medium, e.g. in Ca and phosphate ion concn., influence FA composition; (iii) it is possible to identify an isolated strain to be of a certain origin even if it has grown in a foodstuff and has possibly adapted to various substrates. For (iii), *B. cereus* was inoculated into sterilized boiled rice and a sterilized processed food containing liver. 5 controlled passages in each foodstuff were made. Final bacterial counts were 10^8 - 10^9 cells/g. Results indicate that only minor changes had occurred in the FA composition of *B. cereus*. For rapid identification of *B. cereus* strains, e.g. from food poisoning outbreaks, the determination of FA composition, which takes 72 h, could prove useful. [See 8 1C36-40.] JA

14

Staphylococcal food poisoning aboard a commercial aircraft.

Eisenberg, M. S.; Gaarslev, K.; Brown, W.; Horwitz, M.; Hill, D.

Lancet 2 (7935) 595-599 (1975) [8 ref. En] [Cent. for Disease Control, Dep. of Health and Social Services, Anchorage, Alaska, USA]

On Feb. 3, 1975, 196 (57%) of 344 passengers and 1 steward aboard a commercial aircraft contracted a gastrointestinal illness characterized by nausea, vomiting, abdominal cramps and diarrhoea; 142 passengers and the steward were admitted to hospital. Symptoms developed shortly after a ham and omelette breakfast had been served. An investigation strongly incriminated ham as the vehicle of the outbreak, and the source seems to have been a cook with lesions on his fingers. The attack-rate was 86% for passengers who ate the ham handled by this cook and 0% for passengers who ate ham handled exclusively by another food preparer. Before being served, the ham and omelette had been held at room temp. for 14 h and 10°C for 14.5 h. Specimens of stool and vomitus from ill passengers, left-over food, and the finger lesions of the cook were positive for *Staphylococcus aureus* of identical phage types and antibiotics sensitivities. Preformed enterotoxin was detected in the left-over ham and omelette. AS

15

Clinical aspects of outbreak of staphylococcal food poisoning during air travel.

Effersøe, P.; Kjerulf, K.

Lancet 2 (7935) 599-600 (1975) [7 ref. En] [Dep. of Infectious Disease, Blegdam Hospital, Copenhagen N, Denmark]

See preceding abstr.

16

[Application of heat-stable nuclease for screening foods for the likely presence of enterotoxins.]

Anwendung einer hitzestabilen Nuclease zum Screening von Nahrungsmitteln auf die vermutliche Anwesenheit von Enterotoxinen. [Lecture] Tatini, S. R.

Archiv für Lebensmittelhygiene 26 (1) 24 (1975) [De] [Dep. of Food Sci. & Nutr., Inst. of Agric., St. Paul, Minnesota, USA]

The need for a rapid and inexpensive method for assessing *Staphylococcus aureus* growth in foods and, hence, possible enterotoxin production is discussed. A method is detailed for extraction and assay of extracellular DNase. This occurs in both enterotoxigenic and non-enterotoxigenic strains of *Staph. aureus* and is stable to boiling for 15 min; assay requires 4-5 h. A solution prepared from the food is placed in wells in a toluidine-blue-O-DNA agar plate; the size of the pink zone appearing after incubation is linearly related to log concn. of DNase. Studies of growth of *Staph. aureus* and simultaneous production of enterotoxins A, B, C and D and of DNase in experimentally inoculated

foods (e.g. milk, cream, whey, cheese, smoked cured sausage, ham, bologna) and in commercially prepared foods involved in food poisoning (nonfat dry milk, dried malted milk, butter, ham, genoa sausage) indicated that DNase was detectable amounts of enterotoxins. It is suggested that assay for the heat-stable DNase could be adapted for routine screening of foods for the likely presence of enterotoxins. [See 8 1C36-40.] JA

17

Detection of Clostridium botulinum toxin by local paralysis [in mice] elicited with intramuscular challenge.

Sugiyama, H.; Brenner, S. L.; Dasgupta, B. R. *Applied Microbiology* 30 (3) 420-423 (1975) [6 ref. En] [Food Res. Inst., Univ. of Wisconsin, Madison, Wisconsin 53706, USA]

18

Survival of food poisoning bacteria in cheese.

Tzannetis, S. E.; Papavassiliou, J. *Alimenta* 14 (3) 87-92 (1975) [40 ref. En] [Dep. of Microbiol., Fac. of Med., Univ. of Athens, PO Box 1540, Athens, Greece]

Storage time and temp. effects on the growth of *Staphylococcus aureus*, *Salmonella typhimurium* and *Shigella sonnei* were studied in 2 types of ripe acidic Greek cheeses (pH 4.0), a hard cheese and a White brined cheese. Survival times at 37°, 22° and 4°C were plotted and used to calculate decimal reduction times (DRT). For both types of cheese, death rate increased with storage temp., although initial multiplication of inocula occurred at 37° and 22°C in spite of the low pH, but not at 4°C. Progressive reduction in viable count from initial values of $>10^6$ /g was not associated with a significant drop in pH. 7.8% NaCl did not accelerate death rates of the organisms tested. Survival times were 24-50 days for *Staph. aureus*, 24-92 days for *Shig. sonnei*, and 45->120 days for *Salm. typhimurium*. [See also Tzannetis, S. *Acta Microbiologica Hellenica* (1972) 17 (4) 246-255.] RM

19

The interaction of pH, sodium chloride, nitrite and thermal process on the survival of Cl. botulinum spores. [Lecture]

Jarvis, B. *SIK Report* No. 359, 98-115 (1974) [29 ref. En] [Food Res. Ass., Leatherhead, UK]

Factors influencing survival and growth of *Clostridium botulinum* in pasteurized cured meat products are discussed on the basis of literature data. Aspects considered include: the microflora of cured meats; the presence of low counts of spores with exceptionally high resistance to temp. or inhibitory substances; effects of pH, heat treatment, NaCl and nitrites, singly and in combination; effects of initial spore counts on the results of experimental studies; formation of inhibitory

compounds (including Perigo-type inhibitors) in heated cured meats; effects of NaCl and nitrites on recovery of heated spores; factors influencing toxin formation by *C. botulinum* in cured meats; effects of additives on the microbiological quality of cured meats; and the current trend towards reduction of NaCl and nitrite concn. in cured meats. [See 8 2B18.] AJDW

20

Variables affecting botulinum toxin development and nitrosamine formation in fermented sausages. [Lecture]

Kueper, T. V.; Trelease, R. D.

Proceedings of the Meat Industry Research Conference pp. 69-74 (1974) [En] [Swift & Co., Oak Brook, Illinois 60521, USA]

Studies on factors influencing growth and toxin formation by *Clostridium botulinum*, organoleptic properties, residual nitrite concn. and nitrosamine formation in fermented sausages are briefly described. Variables studied included: nitrite concn. (≤ 300 ppm); nitrate concn. (≤ 1500 ppm); fermentation temp. (50° or 90°F); inoculum level ($\leq 10\,000$ spores/g); pH (5.6 or 5.2); storage conditions ($\leq 80^\circ\text{F}$ for ≤ 3 months); presence or absence of glucose; and use or non-use of a starter culture. Tables of results are given. The results show that: nitrite controlled toxin formation in non-fermented sausages; no toxin was formed in fermented sausages, even in the absence of nitrite; NaNO_2 markedly improves the flavour and appearance of fermented sausage (NaNO_3 also improves flavour and appearance, but to a lesser extent); nitrite disappears rapidly from fermented sausages, but is generated from nitrate during prolonged high-temp. storage. No nitrosamines were detected in any sample. [See 8 2S132.] AJDW

21

Proteolysis of porcine muscle by Clostridium perfringens.

Hapchuk, L. T.

Dissertation Abstracts International, B 36 (1) 150-151: Order no. 75-14748 (1975) [En] [Michigan State Univ., East Lansing, Michigan 48823, USA]

The mechanism by which porcine muscle proteins break down during growth of *Clostridium perfringens* was studied by isolating an enzyme from a culture filtrate of this organism and comparing its action on muscle with that of the intact organism. *C. perfringens* was capable of degrading both sarcoplasmic and myofibrillar proteins, but the isolated enzyme acted mainly on the former only. It is thus presumed that *C. perfringens* also produces other enzymes causing proteolysis. AL

22

Effects of nitrite and ascorbate on botulinal toxin formation in wieners and bacon. [Lecture]

Bowen, V. G.; Deibel, R. H. (United States of America, American Meat Science Association [Symposium])

Proceedings of the Meat Industry Research Conference pp. 63-68 (1974) [6 ref. En] [Food Res. Inst., Univ. of Wisconsin, Madison, Wisconsin 53706, USA]

Studies on the effects of nitrite concn. (0-150 ppm), ascorbate concn. (0-655 ppm) and storage time (≤ 56 days at 80°F) on toxin formation in wieners (inoculated with 1000 *Clostridium botulinum* pqqq spores/g) are described. *C. botulinum* toxin was formed in numerous samples containing ≤ 50 ppm nitrite; toxin was also detected in 1 sample containing 100 ppm nitrite. Ascorbate at the concn. tested had no effect on toxin formation. Studies were also conducted on the effect of nitrite concn. (≤ 160 ppm), ascorbate concn. (≤ 2000 ppm), *C. botulinum* spore inoculum size (875 or 19 500 spores/g) and storage time (≤ 28 days at 80°F) on residual nitrite and ascorbate concn., toxin formation and nitrosamine formation in bacon. Tables of results are given. Nitrite concn. decreased by approx. 96% during smoking, slicing and storage; ascorbate concn. decreased by 48%. Toxin formation was observed in few samples inoculated with 875 spores/g. In the samples inoculated with 19 500 spores/g, nitrite concn. > 120 ppm prevented toxin formation. Ascorbate appeared to promote growth and toxin formation by *C. botulinum*. No nitrosamines were detected in any sample. [See 8 2S132.] AJDW

23

Concentration and purification of microbial enzymes by ultrafiltration.

Walliander, P.; Linko, M.; Linko, Y.-Y.

Kemia-Kemi 2 (8) 373-376 (1975) [15 ref. En] [Dep. of Cehm., Helsinki Univ. of Tech., Helsinki, Finland]

Bacillus subtilis fermentation broth containing α -amylase and protease was conc. by ultrafiltration. In general, enzyme losses by permeation were 0-2% with α -amylase and slightly more with proteases, while 75-80% of the total soluble DM was permeable. Thus, the concn. procedure is also a substantial purification step. During ultrafiltration of heterogeneous fermentation broths a secondary membrane is quickly formed on the actual membrane. This secondary membrane affects both the flux and the permeation characteristics. AS

24

[Aetiology of food poisoning by *Clostridium perfringens*.] Ätiologie der *Clostridium*-perfringens-Lebensmittelvergiftungen.

Prost, E.

Monatshfte für Veterinärmedizin 30 (9) 339-342 (1975) [24 ref. De, en, ru] [Veterinärmed. Fak., Landwirtschaftliche Akad., Lublin, Poland]

Food poisoning caused by *Cl. perfringens* (mainly type A2) is discussed, with reference to: contamination of raw meat with *Cl. perfringens*;

inhibition of *Cl. perfringens* by other microorganisms; destruction of this inhibitory microflora during heat-treatment of foods; survival of *Cl. perfringens* in the alimentary tract; and enterotoxin formation. IN

25

[Methods for detection of staphylococcal enterotoxins.] Methoden zum Nachweis von Staphylokokkenenterotoxinen. [Review]

Sommerfeld, P.; Terplan, G.

Archiv für Lebensmittelhygiene 26 (4) 128-137 (1975) [97 ref. De, en] [Inst. für Hygiene und Tech. der Lebensmittel Tierischen Ursprungs, Univ., Munich, Federal Republic of Germany]

This review is based on a paper presented at the 9th International Symposium organized by the Committee on Food Microbiology and Hygiene of the International Association of Microbiological Societies and held in Kiel in Sept. 1974. It deals with biological methods of detection (monkey feeding test, cat test, tests with other animals, frog semen test); immunological methods of detection (precipitation in liquid media; precipitation in gels, including simple linear immunodiffusion, double linear diffusion, simple radial immunodiffusion, double radial diffusion, and immunoelectrophoretic procedures, including countercurrent electrophoresis and Laurell's electroimmunodiffusion; agglutination reactions, including passive agglutination reactions, and inhibition of haemagglutination; fluorescence serological methods; radioimmunological procedures); and detection of staphylococci and staphylococcal enterotoxins in food, including guidelines on procedure.- SKK

26

Botulism in commercially canned foods.

Lynt, R. K.; Kautter, D. A.; Read, R. B., Jr.

Journal of Milk and Food Technology 38 (9) 546-550 (1975) [37 ref. En] [Div. of Microbiol., FDA, Washington, DC 20204, USA]

Commercially canned foods have had a remarkably good record over the last 45 yr with approx. 775 billion cans of commercially canned foods being consumed with only 4 known deaths until 1971. Since 1971, however, botulinal toxin and/or *Clostridium botulinum* has been found in commercially canned vichyssoise, chicken vegetable soup, peppers, marinated mushrooms, tuna fish, beef stew, and in 41 cans of mushrooms from 20 lots packed by 7 USA and 2 foreign producers. The typical cause of botulism in canned foods is underprocessing which may result from inadequate equipment, improper operating procedures, and scheduled processes which are not appropriate for the actual operating conditions being used. AS

27

Double-antibody radioimmunoassay for staphylococcal enterotoxin C₂.

Robern, H.; Dighton, M.; Yano, Y.; Dickie, N.

Applied Microbiology 30 (4) 525-529 (1975) [14 ref. En] [Food Res. Lab., Health Protection Branch, Dep. of Health and Welfare, Ottawa, Canada]

A sensitive double-antibody radioimmunoassay for staphylococcal enterotoxin C₂ is described. The assay procedure employs anti-rabbit gamma globulin, prepared in goats, to precipitate the antigen-antibody complex of enterotoxin C₂ and anti-enterotoxin C₂. The test is sensitive to 100 pg of enterotoxin. The method was used for detection of enterotoxin C₂ in 4 dehydrated soups implicated in food poisoning. AS

28

A continuing common-source outbreak of botulism in a family.

Horwitz, M. A.; Marr, J. S.; Merson, M. H.; Dowell, V. R.; Ellis, J. M.

Lancet 2 (7940) 861-863 (1975) [9 ref. En] [Bact. Diseases Div., Bureau of Epidemiology, Cent. for Disease Control, Atlanta, Georgia 30333, USA]

In Dec. 1974, 3 cases of botulism occurred in a family; 2 were fatal. In the 3rd patient, the only symptom was dysphagia. *Clostridium botulinum* type B was found in stool specimens from all 3 patients. Home-bottled mushrooms, which were found to contain *Cl. botulinum* type B and its toxin, were believed to be responsible for the outbreak; mushrooms were found at necropsy in the gastrointestinal tracts of both patients who died. Heat treatment of the mushrooms during bottling had been inadequate. AS

29

[Occurrence of staphylococcal enterotoxins in dried milk products.] Zum Vorkommen von Staphylokokken-Enterotoxinen in Trockenmilchprodukten. [Thesis] Meyer, J.

79pp. (1973) [many ref. De, en] Hanover, Federal Republic of Germany; Tierärztliche Hochschule

22 spray-dried and 8 roller-dried whole milk samples, 38 spray-dried and 12 roller-dried skim-milk samples, 60-dried milk baby food samples, 19 feedstuff samples and 11 unclassified samples (from 24 manufacturers) were examined for staphylococcal enterotoxins using various methods of concentration and clean-up and the microslide or single gel diffusion test. Enterotoxins were found in 3 feedstuffs (2 AB and 1 ABC) and 1 roller-dried skim-milk (B) by both diffusion tests. The single gel diffusion test gave false-positive results for 4 baby foods. Model experiments showed that enterotoxin B added to milk and enterotoxin A produced by staphylococci in milk were not inactivated by spray-drying. ADL

30

[Occurrence of coagulase-positive staphylococci and staphylococcal enterotoxins in cultured milk products.] Zum Vorkommen von koagulasepositiven Staphylokokken und Enterotoxinen in sauren Milchprodukten. [Thesis] Kuhnt, J. V.

87pp. (1974) [many ref. De, en] Munich, Federal Republic of Germany; Ludwig-Maximilians-Univ.

53 commercially available cultured milk products, including 35 yoghurts and 5 buttermilks, were tested for coagulase-positive staphylococci (with Giolitti & Cantoni enrichment medium and also Baird-Parker medium) and for staphylococcal enterotoxins A, B, C and D (by a modification of the Casman & Bennet method [Journal of Dairy Science (1968) 51 635-644]). No coagulase-positive staphylococci were isolated. Enterotoxin B was found in a pineapple dessert by the microslide gel diffusion test but not by the single gel diffusion test (suggesting a concn. of 1-2 µg/ml extract). Enterotoxin B recovery in these tests ranged from 33.3 to 50%, depending on initial concn. Heating for 25 min at 85°C reduced the serological activity of enterotoxin B, but incubation and storage had no effect. ADL

31

Study of enterotoxigenic *Clostridium perfringens* type A infection and shedding rates in healthy humans and "Zebu" beef cattle in Brazil.

Brant, P. C.

Dissertation Abstracts International, B 36 (1) 174: Order no. 75-15424 (1975) [En] [Univ. of California, Davis, California, USA]

Studies in Brazil confirmed Californian data that man is an important reservoir of food poisoning *C. perfringens*. 85% of people studied shed *C. perfringens* type A in numbers ranging from 10³ to 5 × 10⁶/g faeces; no effect of age, sex or place of work (restaurant or slaughterhouse) on prevalence of shedding was found, but restaurant workers tended to shed larger numbers. 60% of people examined had internal parasites (prevalence higher in slaughterhouse workers) and these shed fewer *C. perfringens* than non-parasitized workers. Prevalence in cattle was much less than in humans and could be influenced by environmental factors (means of transport, weather). All slaughterhouse workers had antibodies against *C. perfringens* enterotoxin (higher titres in those working on the killing floor), and antibodies are reported for the first time in cattle, but with lower titres than in humans. AL

32

One man's meat, another man's poison. [Lecture] Elias-Jones, T. F.

Institute of Meat Bulletin No. 89, 32-38 (1975) [En] [City Lab., Greater Glasgow Health Board, Glasgow, UK]

The nature and structure of bacteria is described, and the manner in which they can cause food-borne infections, including food poisoning, is indicated. Particular reference is made to the 3 types of food poisoning most commonly encountered in the UK; salmonellosis, *Clostridium welchii* (*Cl. perfringens*) food poisoning and staphylococcal food poisoning. Precautions to reduce the incidence of food poisoning are outlined. AS

33

Influence of the z value of *Clostridium botulinum* on the accuracy of process calculations.

Perkins, W. E.; Ashton, D. H.; Evanchio, G. M.
Journal of Food Science 40 (6) 1189-1192 (1975)
[18 ref. En] [Campbell Inst. for Food Res.,
Campbell Place, Camden, New Jersey 08101, USA]

Thermal death time cans of experimental samples were inoculated with 5×10^5 spores of *Cl. botulinum* strain 213B and heated for varying times at 212°, 223° and 235°F. Contents of swollen cans representative of longest heating times at each temp. where spore survival was indicated were toxin-tested by mouse injection. D values calculated from toxic spoilage were significantly higher than those calculated from pH 7.0 phosphate buffer data at 212°F, slightly higher to slightly lower at 223°F, and significantly lower at 235°F. Slopes of thermal death rate curves (z values) in products tested were therefore only 13.3-14.7°F rather than 18°F. These data suggest that caution should be exercised to ensure that calculated F_0 values (F_{18}^{250}) do not overstate process lethality with respect to *Cl. botulinum* in cans processed at 250°F or below. The negative effect of low z values on process effectiveness was confirmed with a strain of PA 3679 having a z value near 14. Spore survival and spoilage occurred after processes which, on the basis of calculated F_0 values, should have precluded PA 3679 survival. Very accurate spoilage prediction was possible when a z of 14 was substituted for a z of 18 in the lethality calculations. IFT

34

[Bacteriological diagnosis of food poisoning caused by *Bacillus cereus*.]

Polkhovskii, V. A.

Laboratornoe Delo No. 3, 173-174 (1975) [13 ref. Ru] [Belorusskii Univ. im. V. I. Lenina, Minsk, USSR]

B. cereus is best isolated from mixed microflora on Nikodemusz medium (which contains egg yolk); Gram-negative organisms do not grow on this medium due to the presence of ethanol. *B. cereus* is easily distinguished from other spore-formers by its ability to split egg yolk lecithin, which reaction produces an area of precipitation around the colonies. Studies indicated that *B. cereus* isolated from different materials implicated in cases of food poisoning may have been the causal organism. STI

35

Staphylococcal enterotoxin C: solid-phase radioimmunoassay.

Bukovic, J. A.; Johnson, H. M.

Applied Microbiology 30 (4) 700-701 (1975) [6 ref. En] [FDA, Cincinnati, Ohio 45226, USA]

A solid-phase radioimmunoassay test, employing ^{125}I -labelled enterotoxin C and polystyrene tubes coated with 1 ml of sodium sulphate-precipitated antiserum, was used to detect enterotoxin C in condensed milk, Cheddar cheese, custard and ham salad. The assay was sensitive to 1-10 ng toxin/g food; nonspecific inhibitions were 16% or less. AS

36

[Relationships between water activity and microorganisms.] [Review]

Yokozeki, M.

Journal of the Food Hygienic Society of Japan [Shokuhin Eiseigaku Zasshi] 16 (3) 145-152 (1975) [32 ref. Ja] [Tokai Regional Fisheries Res. Lab., 5-5 Kachidoki, Chuo-ku, Tokyo, Japan]

Recent studies of relationships between water activity (a_w) and microorganisms are reviewed with special reference to food poisoning organisms. Topics covered include: measurement of a_w , relationships between growth of microorganisms and a_w , effect of a_w on heat resistance of microorganisms, and production of toxins as related to a_w . TM

37

Influence of pH on the heat inactivation of staphylococcal enterotoxin A as determined by monkey feeding and serological assay.

Humber, J. Y.; Denny, C. B.; Bohrer, C. W.

Applied Microbiology 30 (5) 755-758 (1975) [16 ref. En] [Nat. Canners Ass. Res. Foundation, Washington, DC 20036, USA]

38

Effect of sodium chloride on the staphylococcal growth in milk.

Helmy, Z. A.; Abd-El-Malek, Y.; Mahmoud, A. A.

Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, II 130 (4) 334-342 (1975) [13 ref. En, de] [Agric. Bact. Dep., Fac. of Agric., Cairo Univ., Giza, Egypt]

A study was made of the effects of 0, 2.5, 5, 7.5, 10 and 15% NaCl on the growth of (i) *Staphylococcus aureus* isolated from raw milk, (ii) *Staph. aureus* isolated from Damietta cheese with a 10% NaCl content and (iii) a coagulase-negative staphylococcus. Measurements of bacterial counts, acidity and pH were made after 6, 12, 24, 48, 72 and 96 h incubation at 37°C in a steamed milk medium. Additions of 2.5 or 5% NaCl stimulated growth of (i) and (iii) but levels of 7.5 and 10% NaCl retarded growth and in the presence of 15% NaCl there was only slight growth. With (ii) considerable growth occurred in the presence of 15% NaCl. Adaptation of (i) and (ii) to high salinity resulted in more growth occurring in the presence of 15% NaCl, but the organisms became more sensitive to increases in acidity. The possibility of enterotoxins being present in Damietta cheeses is discussed. MEG

39

[Staphylococci in cheese made from raw milk.]

Mol, H.; Vincentie, H. M.

Tijdschrift voor Diergeneeskunde 100 (18) 991-994 (1975) [16 ref. Nl, en] [Keuringsdienst van Waren te Utrecht, Nijenoord 6, Netherlands]

Coagulase-positive staphylococci ranging from $<10^2$ to $>10^6$ /g were found in 1973 and 1974 in cheese samples made from raw milk. Of 20 strains tested, 8 produced toxin A. In 2 cases where the cheese was suspected as a cause of food poisoning,

the staphylococci numbered $>10^6$ (cheese about 4 wk old) and approx. 1.5×10^4 (mature cheese) per g; in this latter case $>4 \mu\text{g}$ enterotoxin A/100 g was isolated. The 1st cheese was not examined for toxin. ADL

40

Proteins from the sea.

Anon.

British Food Journal 77 (869) 167-169 (1975) [5 ref. En]

Declining UK fish consumption, artificial fish culture, the change from fresh to frozen patterns of fish consumption, and hazards from *Clostridium botulinum* and *Salmonella typhimurium* on fish are discussed in general terms. JRR

41

Enterotoxin producing staphylococci in meat processing plants: origin, biochemical properties, resistance to antibiotics.

Sinell, H.-J.; Kusch, D.; Untermann, F.

European Journal of Applied Microbiology 1 (3) 239-245 (1975) [13 ref. En] [Inst. für Lebensmittelhygiene, Fleischhygiene & Fleischtech., Bitterstrasse 8-12, 1000 Berlin (West)]

3065 staphylococci isolated from slaughtered pigs, meat plant equipment, and various other sources including specimens from human beings, were examined for their biochemical properties as well as phage type pattern and resistance to antibiotics. Staphylococci originating from swine produced enterotoxins to a significantly lesser degree (21%) than strains isolated from clinical specimens (hospital strains, about 40%). Phage group II strains, in particular, were shown to produce enterotoxins very rarely. Strains originating from swine were considerably more resistant to antibiotics than other staphylococcal strains. The resistance to tetracyclines previously found shifted to a marked increase in zinc bacitracin resistance within the past year. This is obviously connected with the use of antibiotics in animal feeds. AS

42

Syntheses and structure of the alkali salts of the ironnitrosylsulfides.

Dymicky, M.; Wasserman, A. E.; Huhtanen, C. N. *Abstracts of Papers, American Chemical Society* 169, AGFD 28 (1975) [En] [E. Regional Res. Cent., USDA, 600 East Mermaid Lane, Philadelphia, Pennsylvania 19118, USA]

Alkali salts of the complexes of iron-nitrosylsulfides, known as Roussin salts, exhibit a very potent activity against *Clostridium botulinum*. These salts may be considered as potential preservative agents for food, particularly for meat. Since the classical methods of preparation of these complexes do not give reproducible results, the procedure of Pavel was modified to improve the formation of the product. Also, a new procedure was developed, involving heating the reactants for about 15 min at 120°C under approx. 15 lb/in^2 (gauge) pressure. Based on extensive analyses and

determination of mol. wt., a new empirical formula for the ether-soluble black complex has been established; $[\text{Fe}_3(\text{NO})_5\text{S}_2] \cdot \text{M}^+ \cdot \text{H}_2\text{O}$ (438.77, $\text{M}^+ = \text{K}^+$). Contrary to the classical concepts, no evidence could be found indicating the presence of univalent iron. Electron paramagnetic resonance, polarographic, IR and other spectral studies indicate that this complex contains Fe^{2+} and Fe^{3+} ions, in a ratio of about 2:1 respectively. AS

43

[Staphylococcal food poisoning on board an aircraft.] Lebensmittelvergiftung durch Staphylokokken an Bord eines Flugzeuges.

Anon.

Fleischwirtschaft 55 (6) 816 (1975) [De]

See FSTA (1976) 8 2C91.

44

Production and stability of hemolysin, phospholipase and lethal toxin by *Bacillus cereus* in foods.

Ivers, J. T.

Dissertation Abstracts International, B 36 (5) 2142-2143; Order No. 75-24200 (1975) [En] [Cornell Univ., Ithaca, New York 14850, USA]

Preliminary studies indicated that *B. cereus* strains 14579, 6464 and 7004 produced different amounts of the enzymes (i) haemolysin, (ii) phospholipase and (iii) lethal toxin after comparable incubation time and growth. A method was developed for estimating activities of the enzymes in foods, and % recoveries of (i)-(iii) in beef, pea and banana were determined. Recoveries of essentially 100% were demonstrated. Further studies with strains 7004 and 14579 indicated that (i), (ii) and (iii) were produced in beef, (i) and (ii) in pea, and (iii) in banana. Effects of food handling practices on the stabilities of (i)-(iii) in foods were examined. All 3 enzymes were inactivated in beef to some extent by heating at 65°C for 3 min; (iii) was more adversely affected than (i) or (ii). All 3 enzymes in beef, pea and banana retained $\geq 50\%$ activity after 24 h at 4°C . Conclusions are that *B. cereus* strain, type of food, temp. and time of incubation, and conditions of food processing and storage are factors all contributing to the special circumstances necessary for *B. cereus* food poisoning to occur. JA

45

Clostridium botulinum in marinated products.

Hauschild, A. H. W.; Aris, B. J.; Hilsheimer, R. *Canadian Institute of Food Science and Technology Journal* 8 (2) 84-87 (1975) [15 ref. En, fr] [Microbiol. Res. Div., Health Protection Branch, Health & Welfare Canada, Ottawa, Ontario, Canada]

Marinated mushrooms in oil were prepared with various amounts of acetic acid (0.1, 0.2, 0.4, 0.8

and 1.0% in final marinade), MARINADE). Botulinal type B toxin was produced in jars with naturally contaminated mushrooms at pH ≤ 5.47 but not at pH ≤ 5.04 . Mushrooms inoculated with spores of *Cl. botulinum* type B allowed toxinogenesis at pH ≥ 4.47 but not at pH 4.62. Only type B spores of *Cl. botulinum* were found in fresh commercial mushrooms. Spore counts by the MPN method were 15/100 g of unwashed mushroom and 41/100 g of lightly rinsed mushrooms. 16 commercial products of marinated vegetables were tested for pH and titratable acid. Results suggest that none of the products would allow growth or toxinogenesis of *Cl. botulinum*. However, the pH of some marinated artichoke hearts was close to the critical level. AS

46

Bacillus cereus food poisoning.

Eade, S. A.

British Food Journal 76 (860) 77, 80 (1974) [En]

Outbreaks of mild food poisoning in the Walton and Weybridge area following consumption of cooked (fried or boiled) long grain rice in Chinese restaurants are reported. The cause of the food poisoning was found to be *B. cereus* which multiplies rapidly on cooked rice which is usually stored in such restaurants at room temp. or above all day and sometimes overnight after boiling. It is noted that 'Chinese Restaurant Syndrome', attributed to excess monosodium glutamate, may be caused by *B. cereus*. DMA

47

[A case of human botulism type E attributable to 'rakefisk'.]

Silset, T. O.; Hole, S.

Norsk Veterinaertidsskrift 87 (7/8) 480-483 (1975) [16 ref. No] [Statens Teknologiske Inst., Akersveien 24 c, Oslo 1, Norway]

A non-fatal case of human botulism attributable to consumption of home-cured trout is described; a second person, who ate only a small quantity of the fish, was only slightly ill. The cured trout responsible for the outbreak was found to contain *Clostridium botulinum* type E, and type E toxin. It is suggested that the fish was contaminated with *Cl. botulinum* during preparation (possibly by cleaning with a vegetable brush previously used for washing potatoes) and that it was then held at too high a temp. and too low a salt concn. AJDW

48

[Some hygiene problems of met. I. Bacteriology.]

Jacques, J.

Industries Alimentaires et Agricoles 92 (9/10) 1095-1100 (1975) [Fr, de, en] [Inst. du Lait, des Viandes et de la Nutr., Univ. de Caen, France]

49

Criteria and procedures for implicating *Clostridium perfringens* in food-borne outbreaks.

Hauschild, A. H. W.

Canadian Journal of Public Health 66 (5) 388-392 (1975) [46 ref. En, fr] [Microbial Div., Food Res. Lab., Health Protection Branch, Health & Welfare Canada, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada]

The minimal requirements for implicating *Cl. perfringens* as the aetiological agent in an outbreak are: symptoms, onset and duration of the illness characteristic for *Cl. perfringens* type A enteritis, plus one of the following criteria: (i) a min. concn. of *Cl. perfringens* cells in epidemiologically incriminated foods; (ii) a min. median *Cl. perfringens* spore count in faecal specimens from patients; (iii) identical serotypes for the spore isolates from most of the faecal specimens; or (iv) identical serotypes for *Cl. perfringens* isolates from foods and from faecal specimens. AS

50

[Detection of botulinus toxin by the immunofluorescence adsorption reaction (IFAR) in experimentally contaminated foods.]

Banasiewicz, R.; Reiss, J.

Roczniki Panstwowego Zakladu Higieny 26 (6) 649-661 (1975) [19 ref. Pl, ru, en] [Ośrodek Wojskowego Inst. Higieny i Epidemiologii, Krakow, Poland]

The IFAR test consists in detection of specific fluorescing globulin of bacterial antigens adsorbed on $AlPO_4$. 3 kinds of fresh meat, 3 of fresh liver, 2 of pate, 1 of goulash, and 17 kinds of fresh, smoked and canned fish were contaminated with botulinus toxin A, and (i) IFAR, (ii) indirect haemagglutination, and (iii) agar gel precipitation tests were used for toxin detection. (ii) and (iii) detected the toxin in all products tested; (i) failed with 13 of the products, including fresh liver, pate, goulash and smoked and preserved fish. (ii) was $1000 \times$ more sensitive than (i) or (iii). SKK

51

Foodborne illness from *Clostridium perfringens*.

[Review]

Walker, H. W.

CRC Critical Reviews in Food Science and Nutrition 7 (1) 71-104 (1975) [many ref. En] [Dep. of Food Tech., Iowa State Univ., Ames, Iowa, USA]

Large numbers of *Cl. perfringens* in food are frequently the cause of a mild form of food illness. The organism is widespread in the environment and occurs normally in soil in the intestinal tract of animals. Sporulation of large numbers of cells in the intestinal tract of animals results in the release of an enterotoxin that produces abdominal cramps and diarrhoea. Animals and serological techniques are used to assay for the enterotoxin. Several selective media and confirmatory tests have been developed for quantitative and qualitative recovery of the organism from foods. Proper sanitation and temp. control can limit occurrence and growth in foods. AS

52

Estimation of populations of *Clostridium perfringens* in a meat medium held at low temperatures.

Fruin, J. T.

Dissertation Abstracts International, B 36 (2) 637: Order No. 75-17198 (1975) [En] [Purdue Univ., Lafayette, Indiana, USA]

Mean survival of vegetative cells of *Cl. perfringens* held in 5 standard diluents for 1 day was 0.066% at -23°C; 20% at 4°C; 0.15% at 37°C. In a meat medium (1 part ground lamb:4.5 parts water), some strains were capable of growth after 1 day at 4°C; after 14 days all strains were stable or had declined below inoculation level. Anaerobic conditions significantly increased culture populations in the meat medium after 7 and 14 days at 4°C compared with aerobic conditions. Storage at -23°C gave the following survivals: after 1 day, 17%; 7 days, 12%; 14 days 6.2%. There were significant differences between strains. Different cooling rates and addition of 10% sucrose did not affect survival during cooling to -23°C; addition of 1.0N glycerol increased survival whereas 0.1N and 5N glycerol did not. AMW

53

Gas chromatographic determination of N-nitrosoamino acids by trimethylsilylation and single-ion mass fragmentography.

Eisenbrand, G.; Janzowski, C.; Preussmann, R.

Journal of Chromatography 115 (2) 602-606 (1975) [15 ref. En] [Inst. for Toxicology & Chemotherapy, German Cancer Res. Cent., Im Neuenheimer Feld 280, D-69 Heidelberg, Federal Republic of Germany]

A method for analysis of trace amounts of (i) N-nitrosoamino acids is described, using N-nitroso compounds of sarcosine, proline and 2-hydroxyproline for the investigation. The procedure is based on trimethylsilylation and gas chromatographic separation of the volatile trimethylsilyl-derivatives of (i), and both flame ionization and single-ion monitoring by GLC-MS for quantitative assessments. The method may be applicable to quantitative detn. of (i) in foods at parts/billion levels. AL

54

Influence of protein supplements on growth of *Staphylococcus aureus* and production of enterotoxins.

Tatini, S. R.; Stein, S. A.; Soo, H. M.

Journal of Food Science 41 (1) 133-135 (1976) [5 ref. En] [Dep. of Food Sci. & Nutr., Univ. of Minnesota, St. Paul, Minnesota 55108, USA]

Influences of soy protein (promine D; SP), fish protein concentrate (FPC) and brewer's yeast or *Torula* yeast on aerobic growth of *Staph. aureus* (Z88) and production of enterotoxins A and D were evaluated in liquid media containing equivalent amounts (1.8%) of protein from each source. *Staph. aureus* grew fast (29 min generation time) and reached a final population of $2-7 \times 10^9$ /ml in yeast media as compared to 50-56 min

generation time and a final population of 2×10^8 /ml in SP or FPC. Production of enterotoxins by *Staph. aureus* was enhanced in yeast media. Enterotoxin A was detectable in unconc. yeast preparations after 6 h and both toxins (A and D) after 12 h incubation. Enterotoxin A was detected only in FPC conc. 50-fold and after 24 h incubation. Neither enterotoxin A nor D was detected in SP conc. 100-fold after 24- or 48-h incubation. Addition of brewer's yeast (2% w/v) to whole milk enhanced production of enterotoxins A and D; there was ≥ 100 -fold higher amount of enterotoxin in milk + yeast as compared to simply milk. IFT

55

[Distribution of enterotoxin-producing strains of *Clostridium perfringens* type A in humans, foods and soils.]

Yasukawa, A.; Okada, Y.; Kitase, T. M.; Miyamoto, S.

Journal of the Food Hygienic Society of Japan [Shokuhin Eiseigaku Zasshi] 16 (5) 313-317 (1975) [20 ref. Ja, en] [Osaka City Inst. of Public Health & Environmental Sci., 21, Tojo-cho, Tennoji-ku, Osaka, Japan]

77% of *Cl. perfringens* strains isolated in connection with food poisoning outbreaks produced enterotoxin whereas only 0.8% of strains isolated from natural environments produced enterotoxin. TM

56

[Characteristics of enterotoxic staphylococci from foods implicated in food poisoning incidents.]

Caserio, G.; Valcamonica, C.; Barluzzi, A.

Latte Nov., 670-672 (1975) [5 ref. It, en] [Istituto di Ispezione degli Alimenti di Origine Animale, Milan, Italy]

9 isolates of staphylococci were from lasagne, 3 from pastries, 2 from Robbiole cheese and 1 from a cooked meat based on pork. The only differences in biochemical characteristics of the isolates were that 3 of them were lecithinase-negative and acid production from mannitol was very variable. Immunodiffusion demonstrated that 11 isolates produced only enterotoxin A, 2 only B, and another 2 produced both A and B. JMD

57

[Detection of staphylococcal enterotoxin B in foods.]

Campanini, M.; Casolari, A.

Industria Conserve 50 (2) 103-105 (1975) [13 ref. It, de, en, fr] [Sta. Sperimentale per l'Ind. delle Conserve Alimentari, Parma, Italy]

Staphylococcal enterotoxin B was detected immunologically by slide agar double-diffusion with a sensitivity of 0.075 µg/ml extract. 100% recoveries were obtained from 30-40 g samples of bouillon cubes and dried milk with 1-2 µg added toxin/g; and 60-80% from salami with 0.1-1.0 µg toxin/g when purification of the extract by column chromatography on Carbowax was omitted. 50% of 0.04 µg/g was recovered from 100 g samples of pasta after chromatographic clean-up. RM

58

Bacterial endotoxins in the environment.

Luzio, N. R. di; Friedmann, T. J.

Nature, UK 244 (5410) 49-51 (1973) [23 ref. En] [Dep. of Physiol., Tulane Univ., School of Med., New Orleans, Louisiana 70112, USA]

The Limulus lysate test of Levin & Bang [Bulletin of the Johns Hopkins Hospital (1964) 115, 265] for Gram-negative endotoxins based on gelation of amoebocyte lysate of the horseshoe crab (*Limulus polyphenus*) by Gram-negative endotoxins, was found to be simple, highly sensitive and also negative with inactivated endotoxins. The tests detected 0.001 µg *Escherichia coli* endotoxin (laboratory standard) in 0.1 ml solution.

Endotoxins were detected in 16 of 18 drinking waters from various US cities (well, river or lake sources); the 2 negative samples were drawn from deep artesian wells. Source surface waters contained 128-400 µg endotoxin/ml and coastal waters 20-400 µg/ml. Purified drinking waters (4 samples) contained 1-10 µg/ml, but Mexico City tap water contained 800 µg/ml. Deionized water was positive, but chemically purified water, some commercial bottled waters and all beer, cola and wine samples tested were negative. Commercial milk samples from New Orleans contained 30-130 µg/ml, with a 16-fold increase in samples held 24 h at room temp. Small endotoxin concn. are not considered hazardous to normal subjects but may become so in some gastrointestinal conditions. Infectivity and mortality following bacterial infections are increased and the presence of endotoxins in milk or water is suggested as a contributory factor to sudden infant death syndromes (rare with breast feeding). ELC

59

[Properties of staphylococci isolated from cows' milk from the viewpoint of milk evaluation.]

Eigenschaften von aus Kuhmilch isolierten Staphylokokken im Hinblick auf die Beurteilung von Milch.

Mayer, S.

Milchwissenschaft 30 (10) 607-608 (1975) [De, en] [Lehrstuhl für Hygiene & Tech. der Milch, Univ., Munich, Federal Republic of Germany]

Of 302 strains of staphylococci isolated from quarters of mastitic cows, 297 were DNAase-positive, 286 produced coagulase, 91 gave a positive egg-yolk test, and 175 gave a positive crystal violet test. Toxin was formed by 45 of the DNAase-positive strains (15 were type D; 11, type A; 10, type B; 4, type C; 1, type E; 3, AD; and 1, BD). Toxin production was much more frequent among the antibiotic-resistant than among the antibiotic-sensitive strains (28% of 93 resistant strains vs. 9.3% of 204 sensitive strains). Information is also presented on phage grouping and haemolysin formation. SKK

60

[Detection of endotoxins of Gram-negative microorganisms in milk by the Limulus test.] Zum Nachweis von Endotoxinen gramnegativer Keime in Milch mit dem Limulus-Test.Terplan, G.; Zaadhof, K.-J.; Buchholz-Berchtold, S. *Archiv für Lebensmittelhygiene* 26 (6) 217-221 (1975) [many ref. De, en] [Inst. für Hygiene und Tech. der Lebensmittel Tierischen Ursprungs, Univ., Munich, Federal Republic of Germany]

No endotoxins were detected by the Limulus test in 29 quarter milk samples taken under aseptic conditions, nor were coliforms present in them. In 53 samples of milk delivered to dairies, a close relationship was found between endotoxin titre and numbers of coliforms. Such a close relationship was found in only 7 of 39 samples of pasteurized milk; in the remainder, the endotoxin titres were relatively appreciably higher. The difference is ascribed to resistance of endotoxins to pasteurization. Endotoxin concn. ranged in raw milk from 0.5 ng to 50 µg/ml and in pasteurized liquid milk, it ranged from 5 to 500 ng/ml. All 40 strains of different Gram-negative bacteria tested gave a positive Limulus test. The view is expressed that endotoxins of Gram-negative microorganisms present in foods present no health hazards to the consumer. The Limulus test is considered suitable for evaluation of the hygienic quality of milk, particularly as a rapid (60 min) method for detection of coliforms before and after pasteurization. SKK

61

Effect of nitrite and erythorbate on growth of *Bacillus cereus* in cooked sausage and in laboratory media.

Raevuori, M.

Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, IB 161 (3) 280-287 (1975) [26 ref. En, de] [Dep. of Food Hygiene, Coll. of Vet. Med., Helsinki, Finland]

B. cereus spores were inoculated (at a calculated level of 5000/g) into sausage mass before cooking. The sausages were kept at 20°C for 48 h before bacteriological and physicochemical analyses were done. Addition of 200 mg/kg sodium nitrite and 500 mg/kg sodium erythorbate before cooking totally prevented growth of 2 strains of *B. cereus* tested. Increasing the amount of erythorbate did not affect the antibacterial properties of the nitrite. The inhibitory effects of different combinations of the additives were also studied in laboratory media. Public health aspects of the use of nitrite and erythorbate as food additives are discussed. AS

62

Immunolectrophoretic detection of staphylococcal enterotoxin A in pork, beef, chicken, and turkey broths.

Humber, J. Y.

Dissertation Abstracts International, B 36 (2) 638: Order no. 75-17724 (1975) [En] [Univ. of Maryland, College Park, (Prince George's County and Baltimore), Maryland, USA]

Pork, beef, chicken and turkey broths, inoculated with *Staphylococcus aureus* strain 100 and incubated for 24 h at 4°C, were analysed for enterotoxin A by immunoelectrophoresis following dialysis against barbital buffer. Approx. 0.07 and 0.06 µg enterotoxin/ml were detected in pork and beef broths respectively. No enterotoxin A could be detected in inoculated chicken or turkey broths. The min. concn. of enterotoxin which can be detected by this method is 0.03 µg/ml. AMW

63

Staphylococcal food poisoning from chicken.
Anon.

British Medical Journal 1 (6002) 164 (1976) [En]

Cold chicken consumed at a party was found to be the source of *Staphylococcus aureus* food poisoning in 22 of the guests. The hot chickens were transported from a fried chicken supplier to the caterer's premises in the cartons in which they came raw from the breeders. After cooling for about 2 h, they were stored overnight in the refrigerator in the same containers. The next afternoon the chickens were sliced and arranged on plates and returned to the same cartons for transport to the party the same evening. Strains of *Staph. aureus* were isolated from the faeces of one of the infected guests and from chicken remains found in the refuse bin 2 days later. All strains had the same phage typing pattern and produced enterotoxins A and D. It is concluded that contamination from an infected person occurred while the chickens were still warm. The dangers of *Salmonella* poisoning arising from the use of the same cartons that had held the raw chickens is also mentioned. MEG

64

Production and thermal inactivation of staphylococcal enterotoxins in meat systems.
Lee, I. C.-H.

Dissertation Abstracts International, B 36 (3) 1126: Order No. 75-20859 (1975) [En]
[Michigan State Univ., East Lansing, Michigan 48823, USA]

Staph. aureus strains 265 and 243 (which produce enterotoxins A and B, respectively) were inoculated into Genoa salami at 10^3 , 10^5 and 10^7 cells/g meat prior to curing, tempering and heating at 38°C for 20 h, 43°C for 2 h, 49°C for 4 h or 54°C for 3 h (all at 90% RH); the sausages were then dried at 12°C and 67-72% RH for approx. 60 days. *Staph. aureus* 265 increased more on the surface than in core samples and higher log cycle increases were recorded at the lower inoculation levels. Heating reduced the increases, and there were decreases during the drying period; however, surface counts remained at $>10^6$ cells/g. After ≥ 8 days, 0.2 µg enterotoxin A/100 g surface sample were detected in samples inoculated with 10^5 or 10^7 cells/g. Similar results were obtained with *Staph. aureus* 243, but no production of enterotoxin B was detected. Thermal stability of crude preparations of enterotoxins A and B were studied at initial concn. of 16-32 µg/ml at 110°,

115.6°, 121.1° and 126.7°C. Thermal stability was higher in beef broth than in brain heart infusion; a dialysable fraction from beef broth with mol. wt. $<12\ 000$ affecting thermal stability of enterotoxin B was isolated. AMW

65

The effects of benzoic acid and nitrite on toxin production by *Clostridium botulinum* type E.
Gudding, R.; Nordal, J.

Acta Agriculturae Scandinavica 26 (1) 47-51 (1976) [16 ref. En] [Dep. of Food Hygiene, Vet. Coll. of Norway, Oslo, Norway]

Sodium benzoate, at a concn. of 5000 ppm, had an inhibitory effect on germination, growth and toxin production by *Cl. botulinum* type E in Robertson's meat broth at pH 5.7. However, 200 ppm nitrite inhibited toxin production more effectively, and may thus be regarded as a better additive for the prevention of type E botulinal toxin production in foods. AS

66

Food poisoning organisms.
Streader, B.

Food Manufacture 51 (4) 37, 39 (1976) [En]

The nature and occurrence of the common food poisoning bacteria are discussed. AS

67

[Detection of staphylococci in foods by culture methods and by detection of nuclease activity.]
Skovgaard, N.

Dansk Veterinaertidsskrift 59 (3) 89-95 (1976) [22 ref. Da] [Inst. for Vet. Mikrobiol. og Hygiejne, Den Kongelige Vet.- og Landbohøjskole, 1870 Copenhagen V, Denmark]

Methods for detection of staphylococci in foods are discussed, with reference to: use of selective culture media; detection of staphylococci on the basis of their DNAase activity, coagulase activity, tellurite resistance, etc.; characteristics and formation of staphylococcal nuclease; microbial decomposition of pre-formed nuclease in foods; and the sensitivity of the nuclease test for staphylococci in foods. A diffusion method (using toluidine blue-O/DNA agar) for detection of staphylococcal nuclease activity in extracts from foods is described. AJDW

68

The excretion of enterotoxin B by *Staphylococcus aureus*: intracellular and extracellular.
Miller, R. D.

Dissertation Abstracts International, B 36 (7) 3236: Order No. 76-1368 (1976) [En]
[Pennsylvania State Univ., University Park, Pennsylvania, USA]

69

[Survival of pathogenic staphylococci in twarog.]
Trawinska, J.

Medycyna Weterynaryjna 31 (9) 533-535 (1975)
[29 ref. Pl, ru, en] [Katedra Higieny Produktow
Zwierzezych, AR, Lublin, Poland]

Samples of freshly pasteurized milk from Lublin dairy factory were inoculated with suspensions of 1 of 10 strains of pathogenic *Staphylococcus aureus* at 10^4 or 10^6 cells/ml, and twarog was made from contaminated and control samples as described in FSTA (1972) 4 4P488. During twarog storage at 20°C, numbers of strains surviving in Twarog from milk inoculated at 10^4 and 10^6 staphylococci/ml respectively were: after 9 days, 10 and 10; after 10 days, 4 and 10; after 11 days, 1 and 8; after 12 days, 0 and 3; no strain survived for ≥ 13 days in any twarog. Twarog pH after 10, 11 and 12 days was respectively 3.9, 3.8 and 3.7. SKK

70

Incidence of clostridia as gas-former in Domiatti cheese.

Fahmy, T. K.; Youssef, L. M.

Agricultural Research Review 52 (7) 233-236 (1974) [9 ref. En] [Dairy Microbiol. Dep., Anim. Production Res. Inst., Agric. Res. Cent., Min. of Agric., Cairo, Egypt]

Samples of market Domiatti cheese were examined for the incidence of *Clostridium* spp. as gas-formers. Clostridia were detected in 30% of samples, with an average count of 54 organisms/g. 2 samples each contained 10 organisms/g although their salt and acidity contents were 5.66-5.78% and 1.76-1.77% respectively. JRR

71

Incidence of staphylococci in the raw market milk and laboratory pasteurized milk.

Juneja, I.; Pal, R. N.

Haryana Agricultural University Journal of Research 4 (4) 304-308 (1974) [13 ref. En] [Dep. of Livestock Production & Management, Haryana Agric. Univ., Hissar, India]

A total of 160 milk samples obtained during the winter and summer from (i) the University farm, (ii) commercial confectioners, (iii) Government livestock farm, and (iv) milk retailers were analysed before and after pasteurization. Raw milk samples from (i)-(iv) contained an average of 7864, 45 327, 60 063 and 30 863 staphylococci/ml respectively vs. 36 029/ml in raw market milk. Pasteurization reduced incidence of staphylococci by 99.96%. In pasteurized milk, 22% of the surviving staphylococci were coagulase-positive vs. 7.3% in raw milk; it is suggested that coagulase-positive staphylococci were more heat resistant. It was concluded that bacteriological quality of raw market milk was poor irrespective of source and season. The HTST method of pasteurization is recommended. MC

72

Market milk products, infected by *Bacillus cereus* in the dairy.

Sogaard, H.

Nordenropacisk Mejeri-Tidsskrift 41 (7) 248-253 (1975) [7 ref. En, Da, De]

As a result of defects caused by *B. cereus* in 2 retail cartons of cream from the same dairy, investigations were carried out to determine the origin of such contamination. *B. cereus* was detected in products from 2 of 6 dairies supervised by the food control office in Odense. At 1 dairy there was only slight contamination in 3 of 191 samples tested; the infection was found only in whole- and low-fat milk, and occurred before pasteurization, but its precise source could not be located. At the other dairy, all products were contaminated and the infection was traced back to the dairy's tankers. The significance of the results is discussed with particular regard to the feasibility of routine screening for *B. cereus*. CDP

73

Quantitation of *Staphylococcus aureus* enterotoxin B by Coulter Counter volume displacement using antibody coated latex.

Olsen, F. W., Jr.; Middaugh, P. R.; Parikh, G. C. *Abstracts of the Annual Meeting of the American Society for Microbiology* 75, 203 (1975) [En]

An automated, semi-micro method is needed to quantitatively determine enterotoxin B (SEB) produced by *Staph. aureus* in foods. SEB in aqueous food extracts functions as an antigen (Ag) and is adsorbed on latex particles (Dow, 2.02 μ m diam.) which have been coated with homologous antibody (Ab). The increased vol. of the Ag-Ab-latex complex is determined with a Coulter Counter. The data were analysed by a programmable mini-computer, Hewlett Packard 9830A, which plots the distribution of particle counts vs. vol. The plot for latex particles has a major peak at channel 6. Latex coated with Ab has a decreased peak height at channel 6. The addition of SEB to form an Ag-Ab-latex complex further increases the particle vol. and decreases the number of latex particles in channel 6. The decrease is proportional to the quantity of SEB/ml in the range 0.01-1.0 μ g/ml. Conc. of SEB from 1.0 to 2.0 μ g/ml are analysed by multi-channel analysis. SEB concn. >2.0 μ g/ml visibly agglutinate the Ab-latex particles. AS

74

Estimating population level of *Clostridium perfringens* in foods based on alpha-toxin.

Harmon, S. M.; Kautter, D. A.

Journal of Milk and Food Technology 39 (2) 107-110 (1976) [11 ref. En] [Div. of Microbiol., FDA, Washington, DC 20204, USA]

75

Staphylococcal growth and enterotoxin production in chicken and chicken salad.

McKinley, T. W.; Henning, W. C.; McCroan, J. E. *Quarterly Bulletin of the Association of Food and Drug Officials of the United States* 38 (1) 56 (1974) [Ej] [Epidemiology Unit, Georgia Dep. of Human Resources, Atlanta, Georgia 30334, USA]

3 batches of chicken were inoculated with an enterotoxin-producing strain of *Staphylococcus aureus* at the rate of 1000, 10 000, and 100 000 staphylococci/g. Each batch contained a different level of saprophytic microorganisms; (i) sterile, (ii) low total count (3000/g), and (iii) high total count (1 400 000/g). After inoculation, portions of the batches were incubated directly, or mixed immediately into salad, or incubated for 6 h or 18 h before mixing into salad. *Staphylococcus* and enterotoxin were determined in duplicate samples of chicken or salad after 24 and 48 h incubation. *Staphylococci* grew luxuriantly in (i) and (ii) and also in salad made from these 18 h after inoculation. *Staphylococcus* growth was generally adversely affected by early mixing of chicken into salad, i.e., mixing at 6 h. Inhibition of staphylococci was severe in (iii) as well as in salad mixed from (iii). Enterotoxin was recovered from (i) and (ii) and from chicken salad mixed from these at 18 h regardless of inoculation size. Enterotoxin was also found after 48 h incubation in one sample of salad which had been inoculated with 100 000 staphylococci/g and mixed into salad at 6 h. Enterotoxin was not found in other samples. [Continued in following abstract.] AS

76

Staphylococcal growth and enterotoxin production in chicken and chicken salad.

McKinley, T. W.; Henning, W. C.; McCroan, J. E. *Quarterly Bulletin of the Association of Food and Drug Officials of the United States* 38 (1) 56 (1974) [En] [Epidemiology Unit, Georgia Dep. of Human Resources, Atlanta, Georgia 30334, USA]

[Continued from preceding abstract.] In general, the greater the saprophytic bacterial contamination and the quicker the chicken was mixed into salad after being inoculated, the less the *Staphylococcus* growth and the less the changes of enterotoxin production. The inhibitory results were attributed to bacterial competition and/or the acetic acid content of mayonnaise. AS

77

[*Bacillus cereus* as an aetiological factor in food poisoning.]

Smykal, B.; Rokoszewska, J. *Roczniki Panstwowego Zakladu Higieny* 27 (1) 47-53 (1976) [15 ref. Pl, ru, en] [Wojewodzka Stacja Sanitarno-Epidemiologiczna, Zielona, Gorza, Poland]

Over a 7-yr period (1964-1971) a total of 533 meat and meat products, 136 soups and meat-based sauces, 1049 samples of milk and milk products and

523 cakes were analysed for presence of *B. cereus*. The organism was found in 13.3, 27.2, 23 and 12.03%, respectively, of the 4 categories of foods (mean, 18.6%). On the basis of findings from 30 volunteers who ate various foods contaminated with 5000-20 000 000 *B. cereus* organisms/ml, it was concluded that the numbers of *B. cereus* organisms was not the sole factor in cases of food poisoning. HBr

78

[Extraction and concentration of *Clostridium botulinum* toxins from specimens.] Extraction und Anreicherung von *Clostridium botulinum*-Toxinen aus dem Untersuchungsmaterial.

Sonnenschein, B.; Bisping, W.

Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, IA 234 (2) 247-259 (1976) [15 ref. De, en] [Inst. für Mikrobiol. & Tierseuchen, Tierärztliche Hochschule, Hanover, Federal Republic of Germany]

Artificially contaminated canned beans were used to develop a suitable procedure for extraction and concentration of botulinum toxins A-E. The procedure consisted of 4 steps: (i) canned beans were diluted 1:2 with 0.1M phosphate buffer, pH 6.0; (ii) the diluted material was homogenized in an 'Ultra-Turrax' homogenizer for 20 s; (iii) the homogenized material was centrifuged at 4000 rev/min for 30 min; (iv) 15 ml supernatant was concentrated using a 'Millipore ultrafiltration chamber' (with a membrane capable of excluding all material with a mol. wt. >25 000). A pressure of 1.5 atm was applied until the terminal vol. was 0.5 ml. After extraction and concentration, the samples were assayed for botulinum toxin in mice. Using this assay the concentrations of the 5 toxins were shown to be as follows: type A toxin, 19.0-fold; type B toxin, 14.8-fold; type C toxin, 20.9-fold; type D toxin, 28.2-fold; and type E toxin, 112.2-fold. AS

79

Thermal inactivation of staphylococcal enterotoxins A and D in certain foods.

Soo, H. M.; Tatini, S. R.; Bennett, R. W.

Abstracts of the Annual Meeting of the American Society for Microbiology 74, 14 (1974) [En] [Univ. of Minnesota, St. Paul, Minnesota, USA]

Staphylococcal enterotoxins A and D were heated separately in milk, skim-milk, cream, cheese whey and sausage in the range of 72-121°C at 1-0.1 µg toxin/ml or 100 g. Toxins were also heated in milk at 143.3°C for 9 s in a 'Spiratherm' unit. Toxin concn. was determined before and after heating by a microslide immunodiffusion method.

Pasteurization (72°C for 15 s) showed practically no true loss of serological activity in milk, cream or skim-milk as indicated by the urea-treated samples: 70-100% serological activity recovered with 1 µg of toxin A or D/ml. In sausage (1 µg toxin A/100 g) heated to reach 100°C, 5% of serological activity was

recovered. In milk heated at 143.3°C for 9 s, 4% and 8% of 0.05 and 0.5 µg toxin A/ml, respectively and 10% of 0.05 µg toxin D/ml were recovered. With 1 µg toxin A/ml in milk, complete inactivation of toxin was observed between 15 and 20 min at 121°C and between 40 and 50 min at 110°C. At 100°C, 4% of serological activity was recovered after 60 min heating. Therefore, it is rather difficult to thermally inactivate toxins without altering the flavour and/or texture of foods. AS

80

[Incidence of *Clostridium botulinum* in Norwegian fish farming establishments.]

Tjaberg, T. B.; Hastein, T.

Norsk Veterinærtidsskrift 87 (11) 718-720

(1975) [11 ref. No] [Norsk Inst. for Næringsmiddel forskning, Postboks 50, 1432 As, Norway]

A total of 152 samples of fish from fish-farming establishments in Norway was examined for *Clostridium botulinum* and its toxin. Cl. botulinum type E toxin was detected in 17 samples; Cl. botulinum type E was isolated from 4 samples. Possible sources of contamination with Cl. botulinum are discussed, and the food hygiene significance of the occurrence of Cl. botulinum toxin in fish is considered, with special reference to the danger of consumption of raw fish. AJDW

81

Validity of the coagulase test in the identification of *Staphylococcus aureus*.

Sperber, W. H.; Tatini, S. R.

Abstracts of the Annual Meeting of the American Society for Microbiology 74, 12 (1974) [En]

[Pillsbury Co., Minneapolis, Minnesota, USA]

Procedures for isolation of Staph. aureus from foods, including the AOAC procedure, regard a positive coagulase test reaction as the definitive identification of Staph. aureus. Many of these procedures specify that any degree of clotting in the coagulase plasma, often interpreted progressively from 1+ through 4+, should be considered as a positive reaction. However, it was found that organisms other than Staph. aureus, but isolated from the usual Staphylococcus-selective media, give a 1+ through 3+ coagulase test reaction, using rabbit coagulase plasma EDTA (RCP). Tests for characteristics such as anaerobic glucose and mannitol fermentation, thermonuclease production and lysostaphin sensitivity proved that these organisms were not Staph. aureus and often were not even Staphylococcus sp. Furthermore, nearly all of the Staph. aureus strains studied produce a very firm (4+) clot in RCP. Therefore, in the use of RCP, only a 4+ reaction can be considered as a positive identification of Staph. aureus. Cultures yielding a 1+ through 3+ reaction cannot be considered Staph. aureus unless confirmed by other characteristics. Pig coagulase plasma EDTA or a mixture of it and RCP appears to be more suitable for use in the coagulase test than RCP alone. AS

82

Preliminary studies on comparison of fluorescent antibody (FA) technique to the Association of Official Analytical Chemists (AOAC) procedure for identification of coagulase positive staphylococci.

Bulawka, C. E.

Abstracts of the Annual Meeting of the American Society for Microbiology 74, 12 (1974) [En] [FDA, Baltimore, Maryland, USA]

A total of 87 samples of refrigerated biscuit dough, chocolate cakes with cream-filling, and fresh crabmeat were examined for coagulase-positive staphylococci. Growth-positive tubes of Trypticase Soy Broth (TSB) with 10% salt were streaked onto Baird-Parker Agar and culturally confirmed for coagulase-positive staphylococci according to AOAC procedures. The same growth-positive TSB tubes were fixed onto multiwell slides and stained with DIFCO FA- *Staphylococcus aureus* conjugate. Positive slides showed intensely fluorescing cocci (+3 to +4 fluorescence). 550 TSB tubes were examined for coagulase-positive staphylococci by AOAC and FA methods. Of the total number of TSB tubes examined, 51% were negative upon completion of cultural tests. Of the total number of TSB tubes examined by FA, 1.6% of positive FA slides were culturally negative and 3.2% of negative FA slides were culturally positive. AS

83

[Incidence of heat-resistant *Clostridium perfringens* in faeces of healthy persons, serotyping of isolates, and food poisoning caused by new serotypes.]

Itoh, T.

Annual Report of Tokyo Metropolitan Research Laboratory of Public Health 24, 7-39 (1972, publ. 1973) [62 ref. Ja, en] [Div. of Bact. I, Dep. of Microbiol., Tokyo Metropolitan Res. Lab. of Public Health, Tokyo, Japan]

An extensive investigation was carried out in Tokyo in 1966-1970 of the occurrence of heat-resistant strains of Cl. perfringens in the faeces of healthy persons. Attempts were made to serotype the strains isolated; a considerable number were classified as TW serotypes. A study of food poisoning outbreaks in Tokyo indicated that in the period 1968-1970, 3 outbreaks, involving 518 cases, were caused by TW strains. An experimental study was also made in an attempt to establish a method for assessing the heat resistance of Cl. perfringens strains isolated from foods. Enrichment in thioglycolate followed by incubation in the spore-forming media of Kim et al. [Applied Microbiology (1967) 15, 871] or of Duncan & Strong [Applied Microbiology (1968) 16, 82] is recommended. [From En summ.] JA

84

[Purification of staphylococcal enterotoxin E.]

Terayama, T.; Yamada, S.; Igarashi, H.; Ushioda, H.; Zen-Yoji, H.

Annual Report of Tokyo Metropolitan Research Laboratory of Public Health 25, 9-15 (1974, publ. 1975) [20 ref. Ja, en] [Tokyo Metropolitan Res.

Lab. of Public Health, 24-1, Hyakunincho 3 chome, Shinjuku-ku, Tokyo 160, Japan]

85

Influence of starch source on sporulation and enterotoxin production by *Clostridium perfringens* type A.

Labbe, R.; Somers, E.; Duncan, C.

Applied and Environmental Microbiology 31 (3) 455-457 (1976) [9 ref. En] [Food Res. Inst., Univ. of Wisconsin, Madison, Wisconsin 53706, USA]

In 16 different starch preparations tested, *Cl. perfringens* NCTC 8798 yielded max. sporulation and enterotoxin formation when ICN-soluble starch [ICN Pharmaceuticals, Cleveland, Ohio] was included in Duncan and Strong sporulation medium [Applied Microbiology (1968) 16, 82-89]. In general, soluble starches were better than potato, corn, or arrowroot starch with regard to these 2 parameters. AS

86

An outbreak of *Bacillus cereus* food-poisoning in Finland associated with boiled rice.

Raevuori, M.; Kiutamo, T.; Niskanen, A.; Salminen, K.

Journal of Hygiene 76 (3) 319-327 (1976) [23 ref. En] [Dep. of Food Hygiene, Coll. of Vet. Med., 00550 Helsinki 55, Finland]

A food-poisoning outbreak caused by *B. cereus* occurred in a Finnish industrial plant in Jan. 1975. 18 of 36 persons who ate a lunch including boiled rice, meat and vegetables became ill. The disease pattern was similar to previously reported short incubation time *B. cereus* food-poisonings associated with cooked rice. The median incubation time was 2 h, the main symptoms being nausea, abdominal pain and vomiting. Rice and certain seasonings were the contaminated raw materials. Gas chromatographic fatty acid analysis of a bacterial cell [FSTA (1976) 8 2C87] was used as a diagnostic method as well as to identify a certain strain of *B. cereus*. To prevent further outbreaks, rice and spices of good microbiological quality should be chosen. Temp. $>10^{\circ}\text{C}$ should be avoided during storage of boiled rice. The whole portion of meal should be prepared at the same time in order to avoid the biphasic temp. curve which enables *B. cereus* to grow in rice. AS

87

[Survey of contamination by *Staphylococcus aureus* in commercial European-type cakes and demonstration of enterotoxin production by isolates.]

Terayama, T.; Igarashi, H.; Ushioda, H.; Saito, K.; Inaba, M.; Zen-Yoji, H.

Annual Report of Tokyo Metropolitan Research Laboratory of Public Health 24, 1-5 (1972, publ. 1973) [5 ref. Ja, en] [Div. of Bact. I, Dep. of Microbiol., Tokyo Metropolitan Res. Lab. of Public Health, Tokyo, Japan]

During Jan.-March 1971, a survey was made of *Staph. aureus* contamination in 1077 samples of European-type cakes obtained from confectioners

in Tokyo. *Staph. aureus* was isolated from 24 samples. The 24 isolates were tested for enterotoxin production; 23 were capable of producing toxin. The predominant enterotoxin was D, followed by C and A. AS

88

Effect of nitrite and ascorbate on formation of botulinum toxin in cured meats.

Bowen, V. G.; Deibel, R. H.

Abstracts of the Annual Meeting of the American Society for Microbiology 74, 13 (1974) [En] [Univ. of Wisconsin, Madison, Wisconsin, USA]

Nitrite prevents formation of *Clostridium botulinum* toxin in cured meat products. The effect of added ascorbate on the efficacy of nitrite inhibition of toxin formation was examined in wieners and bacon that were inoculated with *Cl. botulinum* spores and temp. abused. Toxicity tests in mice revealed that toxin production was inhibited by nitrite at levels $>50\text{ }\mu\text{g/g}$ of wiener. Ascorbate at levels of 0, 105, and 655 $\mu\text{g/g}$ did not decrease the effectiveness of the nitrite inhibition nor did ascorbate potentiate it. Mouse toxicity tests of bacon emulsions indicated that levels of ascorbate $>500\text{ }\mu\text{g/g}$ were associated with a greater incidence of toxic samples, thus indicating a definitive impairment of the nitrite inhibition of *Cl. botulinum* toxin production. AS

89

Effect of starter culture on staphylococcal enterotoxin and thermonuclease production in dry sausage.

Niskanen, A.; Nurmi, E.

Applied and Environmental Microbiology 31 (1) 11-20 (1976) [31 ref. En] [Tech. Res. Cent. of Finland, Food Res. Lab., Biologink. 1, SF-02150 Espoo 15, Finland]

Different amounts of enterotoxin A-, B-, and C_1 -producing staphylococci were added to dry sausage, either alone or with a starter culture (micrococci and lactobacilli). The sausages was examined after 0, 3, 7, 14 and 30 days for staphylococci, micrococci and lactobacilli, and measurements were made of water activity, pH, enterotoxin, and thermostable nuclease. In the absence of starter culture measurable amounts of enterotoxin A were formed in a 200-g sample of dry sausage in 3 days, the level of *Staphylococcus aureus* infection being $>10^6$ cells/g. Enterotoxin B was not found, although the total number of staphylococci was $>10^8$ cells/g. Enterotoxin C_1 was observed when the staphylococcal count was about 8×10^7 cells/g, but was no longer detectable after 7 days. The starter culture prevented the production of enterotoxin A in all cases investigated. By construct, a very high-level inoculation of an enterotoxin C_1 -producing strain gave a positive result after 3 days of incubation even in the presence of a starter culture. Heat-stable nuclease was observed in all sausages to which enterotoxin-producing staphylococci were added. The cell count determined in a sample of sausage had no definite correlation with the thermonuclease activity of the sample. AS

90

Use of the Limulus lysate endotoxin test to assess the microbial quality of ground beef.

Jay, J. M.

Abstracts of the Annual Meeting of the American Society for Microbiology 74, 13 (1974) [En] [Wayne State Univ., Detroit, Michigan, USA]

Since the Limulus lysate test (LLT) is an excellent test for bacterial endotoxins in clinical specimens, an attempt was made to adapt it to food products. Ground beef samples were held at 5°C for ≤ 13 days. Aqueous extracts of beef were made using pyrogen-free distilled water and serial dilutions were subjected to the LLT, employing Difco's reagent. Results were recorded as the highest dilution of meat extract causing gel formation. 12 samples of retail-store, good quality ground beef tested on day of purchase had endotoxin titres of 10^1 - 10^2 . As spoilage occurred, endotoxin titers increased linearly to 10^5 . Rising endotoxin titers were always related to increasing numbers of Gram-negative bacteria and not to meat ageing per se as determined by meat storage in gas-impermeable plastics bags. Log 5.0 of viable Gram-negative cells appeared to be the min. for a positive test. Endotoxin titres of 10^3 were generally attained when the Gram-negative viable counts/g of meat on VRB agar reached log 7.0. This method detects the presence of both viable and non-viable Gram-negative organisms. Evidence to date suggests that a 10^5 dilution of extract from good quality ground beef should give a negative lysate test and thus serve as a rapid screening test of microbial quality. AS

91

The growth of enterotoxigenic staphylococci in chicken salad.

Keoseyan, S. A.; Bennett, R. W.; Amos, W. T. *Abstracts of the Annual Meeting of the American Society for Microbiology* 74, 14 (1974) [En] [FDA, Washington, DC, USA]

Enterotoxin production by *Staphylococcus aureus* strains 743 (type A), 778 (type B), 137 (type C), 494 (type D), and 485 (types A, B and D) was investigated in chicken salad. The organisms were added to laboratory-prepared chicken salad at a dilution of 3.5×10^4 organisms/g and incubated for 15, 18, 21, 24, and 27 h at 35°C. A similar set of uninoculated chicken salad samples was prepared to determine the total count of indigenous microflora. Enumeration and extraction of salad followed by serological assay for enterotoxin(s) utilizing a microslide gel diffusion method were performed on each analytical unit. Uninoculated samples ranged in total count from 8.3×10^7 to 8.1×10^8 organisms/g with no detection of enterotoxin types A, B, C or D. Strain 743 produced detectable amounts of enterotoxin within 18 h with a count of 1×10^7 staphylococci/g. Strain 778 produced detectable amounts of enterotoxin within 24 h with a count of 9×10^7 staphylococci/g. Strain 485 produced detectable amounts of enterotoxins B and D within 18 h with a count of 1.7×10^8 staphylococci/g, and enterotoxin A was detected at 21 h with a count of

2.3×10^8 staphylococci/g. Strains 137 and 494 produced enterotoxins C and D, respectively, at a later time. AS

92

Improved methods for enumerating staphylococci and detecting staphylococcal enterotoxin in meat foods. [Lecture]

Julseth, R. M.; Dudley, R. P.

Proceedings of the European Meeting of Meat Research Workers 19 (Part II) 511-523 (1973) [14 ref. En, fr] [Res. & Development, G. A. Hormel & Co., Austin, Minnesota 55912, USA]

An improved method for detn. of coagulase-positive staphylococci in foods is described, based on incorporation of porcine and rabbit blood plasmas in the plating medium; staphylococcal coagulase, if present, reacts with the plasma to form an opaque zone (the 'fibrin halo') around each *Staph. aureus* colony. Results can be read in 48 h, rather than 72 h with the standard tube method. A modification to the reversed-passive haemagglutination test for staphylococcal enterotoxins is described, in which interfering proteins in the meat extract are separated by precipitation with $(\text{NH}_4)_2\text{SO}_4$. [See FSTA (1976) 8 9S1589.] AJDW

93

Screening for staphylococcal enterotoxins in foods. [Lecture]

Tatini, S. R.; Cords, B. R.; Gramoli, J.

Food Technology 30 (4) 64, 66, 70, 72-74 (1976) [16 ref. En] [Dep. of Food Sci. & Nutr., Univ. of Minnesota, St Paul, Minnesota 55108, USA]

Heat-stable deoxyribonuclease (DNAase) was used as the indicator in a screening procedure for *Staphylococcus aureus* and probable enterotoxins in products such as Cheddar cheese, milk, butter, dried skim-milk and dried malted-milk, ground beef, Genoa sausage and Thuringer (fermented sausage). The main criteria for an indicator, rapid assay, production by all strains, production limited to *Staph. aureus*, production in detectable amounts, and resistance to processing conditions were shown by heat-stable DNAase. The procedures for extraction and assay of the thermonuclease and its production in the above food products are reviewed. [See also FSTA (1975) 7 10C374.]

LMB

94

Staphylococcal enterotoxins - detection in food. [Lecture]

Bergdoll, M. S.; Reiser, R.; Spitz, J.

Food Technology 30 (5) 80, 82, 84 (1976) [13 ref. En] [Food Res. Inst., Univ. of Wisconsin, Madison, Wisconsin 53706, USA]

95

[Lecithinase and toxin production by strains of the genus *Bacillus*.] Lecithinase- und Toxinbildung durch Stämme der Gattung *Bacillus*.
Berkel, H.; Hadlok, R.

Archiv für Lebensmittelhygiene 27 (2) 63-65 (1976) [31 ref. De, en] [Inst. für Tierärztliche Nahrungsmittelkunde, Justus-Liebig Univ., Giessen, Federal Republic of Germany]

A critical examination of literature on the toxicity of aerobic spore-forming bacteria, especially *B. cereus*, indicated that the occurrence of toxins could depend on the ability of the bacteria to produce lecithinase. This opinion was confirmed by earlier work by the authors on 'non-*B. cereus* intoxications'. A continuation of this work with 17 strains of *B. cereus* and 20 each of *B. licheniformis*, *B. subtilis* and *B. pumilus* is reported. A majority of the strains were found to be lecithinase-positive. JA

96

Heterogeneity of *Staphylococcus aureus* enterotoxin B as a function of growth stage: implications for surveillance of foods.

Chesbro, W.; Carpenter, D.; Silverman, G. J. *Applied and Environmental Microbiology* 31 (4) 581-589 (1976) [14 ref. En] [Dep. of Microbiol., Univ. of New Hampshire, Durham, New Hampshire 03824, USA]

Staph. aureus was grown in a fermentor under controlled conditions of pH, oxygenation, and temp., while the higher-mol. wt. products of its growth were continuously removed across ultrafiltration membranes. These products were examined by single and double gel diffusion and immunoelectrophoresis against a variety of available anti-enterotoxin B antisera. All antisera examined were polyvalent for *Staph. aureus* antigens. However, 2 electrophoretically distinct proteins were the major reactants with the antisera. One of these was present in early- to mid-log phase cultures. After mid-log growth was achieved, both were present but in continuously changing proportions. This observation was repeated with a variety of growth conditions and media. A significant part of the physicochemical heterogeneity of enterotoxin B observed over the past 20 yr is thus correlated with the growth phase of the organism. Taken together, these facts are used to argue for a 2-step rationale for the detection of food-borne staphylococcal disease: (i) screening for a presumptive hazard by analysis for any antigen, toxin, or enzyme of *Staph. aureus* in a foodstuff, and (ii) confirmation of the hazard by identifying the presence of an enterotoxin using a combination of physicochemical and serological techniques. AS

97

[Occurrence of pathogenic microorganisms in Cottage cheese.]

Maleszewski, J.; Bachryj, F.; Bawlik, I.; Borowiak, M.; Czarnowska, W.; Chybowski, J.; Dziurawik, Z.; Frasniewicz, B.; Glowacki, M.; Jedrzejowska, H.; Klos, J.; Koc, T.; Krzeminska, B.; Lewicka, J.; Lichocinska, H.; Maciaszek, A.; Smykal, B.; Wilczynska-Stelmach, W.

Roczniki Panstwowego Zakladu Higieny 27 (2) 147-151 (1976) [13 ref. Pl, ru, en] [Zaklad Badania Zywnosci i Przedmiotow Uzytku Panstwowego Zaklad Higieny, Warsaw, Poland]

995 samples of twarog cheese were examined. Coagulase-positive staphylococci were found in 7.8% of 587 high-fat factory cheeses, 6.6% of 202 low-fat factory cheeses and 9.9% of 306 farm cheeses. Haemolytic streptococci were found only in 7.3% of the low-fat factory cheeses, and *Pseudomonas aeruginosa* in 2.5% of the high-fat cheeses. *Escherichia coli* were found in about 40-45% of each group of samples. No *Salmonella* organisms were found. Contamination with moulds was most frequent in the farm cheeses. ADL

98

Growth of *Staphylococcus aureus* and production of enterotoxins in pepperoni.

Tatini, S. R.; Lee, R. Y.; McCall, W. A.; Hill, W. M.

Journal of Food Science 41 (2) 223-225 (1976) [14 ref. En] [Dep. of Food Sci. & Nutr., Univ. of Minnesota, St. Paul, Minnesota 55108, USA]

Growth of enterotoxigenic *Staph. aureus* and production of enterotoxins (A and D) during curing, smoking and drying of pepperoni were evaluated by inoculating raw ingredients with 10^4 , 10^5 , or 10^6 *Staph. aureus*/g and processing by 2 approved (USDA) methods: (i) product smoked for 1 h and heated to 40°C within 5 h and (ii) smoked for 1 h followed by heating to 49-50°C within 11 h. The pepperoni sticks were then dried at 10-12°C for 4-5 wk. Total viable plate count (plate count agar), plate count on rogosa's SL medium (RSL) and *Staph. aureus* count on Baird-Parker medium (BP) were determined. The initial total plate count/g varied between 3×10^5 and 3×10^7 and reached about 10^6 - 10^7 during processing or drying. Though there was an increase of about 2-4 log in the RSL during smoking and/or drying there was no substantial change in pH (6.0-6.2 initial and 5.9-6.2 final). Under these conditions, there was little or no increase in *Staph. aureus* (BP) count during curing, smoking or drying. Smoking contributed substantially to inhibition of *Staph. aureus* growth because unsmoked product, under the same conditions, showed a 1-2 log increase in *Staph. aureus* with (ii). Even though *Staph. aureus* reached a population of 4×10^7 /g in pepperoni processed without smoking by (ii), detectable amounts of enterotoxins were not present in 100 g. Pepperoni processed with smoking by either method is not likely to be a health hazard from staphylococcal enterotoxins. IFT

99

The survival of potential food poisoning bacteria in vacuum-packaged meat. [Lecture]

Patterson, J. T.; Gibbs, P. A.

Proceedings of the European Meeting of Meat Research Workers No. 20, 187-189 (1975) [13 ref. En, fr, de, ru] [Dep. of Agric. for N. Ireland, Newforge Lane, Belfast BT9 5PX, UK]

Survival of *Staphylococcus aureus* (3 strains), *Salmonella muenster*, *Salm. dublin* and *Clostridium welchii* on vacuum-packaged beef was studied. 25 g samples of beef were inoculated with one of the bacterial strains studied (2 levels of inoculum being studied with each strain), vacuum packaged in nylon/polyethylene film, and stored at 0-2°C for ≤8 wk. Tables of results are given. The results show that 2 of the 3 strains of *Staph. aureus* survived for 8 wk; however, counts decreased during storage, and no multiplication during subsequent holding of the packs at 15°C was observed. *Salmonellae* survived for ≤6 wk; no multiplication during subsequent holding at 15°C was observed. *Cl. welchii* survived for 8 wk; multiplication was observed during subsequent holding at 15°C in the sealed pack. [See FSTA (1976) 8 10S1831.] AJDW

100

Recovery of staphylococcal enterotoxin from foods by affinity chromatography.

Genigeorgis, C.; Kuo, J. K.

Applied and Environmental Microbiology 31 (2) 274-279 (1976) [32 ref. En] [Dep. of Epidemiology & Preventive Med., School of Vet. Med., Univ. of California, Davis, California 95616, USA]

Food extracts such as Italian style dry salami of various ages, Cheddar cheese and also fresh milk were exposed to an insoluble matrix tagged with specific anti-enterotoxin B serum by the following method. Immunoglobulin G antibodies were attached to CNBr-activated Sepharose 4B at pH 8.5 by mixing at room temp. for 2 h. Sepharose-antibody complex (1 ml) specifically recovered 0.1-30 µg enterotoxin B from 400 ml food extract (100 g food) after mixing for 2 h at 4°C. The Sepharose-antibody-toxin complex was washed with 0.02M phosphate-buffered saline at pH 7.2, and the toxin was dissociated by 2-4 ml of 0.2M HCl-glycine + 0.5M NaCl buffer at pH 2.8. The recovered enterotoxin was free of interfering food components and could be detected serologically. The enterotoxins produced in milk, cheese and the dry salamis were nontoxic. Work to couple antibodies A, B, C, D, and E to Sepharose to recover all 5 toxins in 1 step is under study. LMB

101

Serological methods for the assay of staphylococcal enterotoxins.

Holbrook, R.; Baird-Parker, A. C.

Technical Series, Society for Applied Bacteriology No. 8, 107-128 (1975) [43 ref. En] [Univ. of

Res. Lab., Colworth House, Sharnbrook, Bedford, UK]

A method is described for extraction of staphylococcal enterotoxin from foods, and several methods for serological assay are outlined.

Effective methods must be capable of detecting ≤0.01 µg/ml if pre-concentration is to be omitted in the interests of speed, and such methods are not yet generally available. JRR

102

[Protection from noxious substances in food as a duty of food control.] Der Schutz vor Schadstoffen in der Nahrung als Aufgabe der Lebensmittelüberwachung.

Sperlich, H.

Ernährungs-Umschau 22 (10) B37-B39; (11) B41-B43 (1975) [36 ref. De] [7000 Stuttgart 1, Postfach 1219, Federal Republic of Germany]

Possible sources of contamination of food are considered with particular reference to the occurrence of Pb, Hg, radioactive substances, mycotoxins and carcinogenic hydrocarbons on materials destined for human consumption. The practice by which bulk raw food materials are transported in containers previously used for transport of non-edible materials is strongly condemned, and an example, contamination of bakery flour with lime, is quoted. Atmospheric Pb contamination by petrol fumes is considered in the light of regulations reducing the permitted level in petrol from 0.6 to 0.4 g/l. The fallacy involved in the assumption that materials of natural origin are harmless is emphasized, with reference to toxins produced by microorganisms, e.g. botulinum toxin and aflatoxin. Introduction of legislation limiting the permissible content of aflatoxin in materials for human consumption to a value of 10 µg/kg is expected. The intake of noxious substances in small amounts, e.g. carcinogens and potential carcinogens, must be viewed in the context of the total exposure situation, since such items cannot be completely excluded from the diet. BDH

103

Food- and waterborne disease outbreaks on passenger cruise vessels and aircraft. [Lecture]

Merson, M. H.; Hughes, J. M.; Lawrence, D. N.; Wells, J. G.; Agnese, J. J. d'; Yashuk, J. C.

Journal of Milk and Food Technology 39 (4) 285-288 (1976) [14 ref. En] [Bureau of Epidemiology, Cent. for Disease Control, Public Health Service, US Dep. of Health, Education & Welfare, Atlanta, Georgia 30333, USA]

This paper presents data collected from outbreaks of foodborne and waterborne disease that occurred in the period 1970-1975 aboard passenger cruise vessels and aircraft. Outbreaks of illness on passenger cruise vessels were caused by *Shigella flexneri*, *Salmonella*, and *Vibrio parahaemolyticus*. Vehicles for the aetiological agents were water, multiple foods, seafood cocktail, shrimp and lobster. *Staphylococcus aureus* caused 2 outbreaks of foodborne illness on aircraft during the same period. Vehicles were custard and ham. AS

104

Collaborative study of the serological identification of staphylococcal enterotoxins by the microslide gel double diffusion test.

Bennett, R. W.; McClure, F.

Journal of the Association of Official Analytical Chemists 59 (3) 594-601 (1976) [15 ref. En] [Div. of Microbiol., FDA, Washington, DC 20204, USA]

A collaborative study was conducted, using the microslide gel double diffusion test for enterotoxin detection, by determining the staphylococcal enterotoxigenicity of 7 strains of *Staphylococcus aureus*. 2 strains produced staphylococcal enterotoxin A (SEA), 2 strains produced staphylococcal enterotoxin B (SEB), 1 strain produced both SEA and SEB, and 2 strains produced neither SEA nor SEB. To ascertain the effectiveness of this method for the detection of enterotoxins, strains of enterotoxigenic staphylococci which produced small or relatively large amounts of toxin were used in the study. The cultures were grown on semi-solid brain-heart infusion agar and the culture fluids were assayed serologically for SEA and SEB. The qualitative results of this study by 16 collaborators indicated that this technique, in general, demonstrated a high degree of specificity as well as simplicity and reproducibility in the identification of the enterotoxins. Of the 16 participating laboratories, 14 reported the correct diagnoses for the 7 staphylococcal strains studied and 2 laboratories determined 6 of the 7 strains correctly. Based on these results, this method has been adopted as official first action: AS

105

The bacteriological quality of boiled and fried rice from Chinese restaurants in relation to *Bacillus cereus* food poisoning.

Beckers, H. J.

Archiv für Lebensmittelhygiene 27 (2) 66-68 (1976) [18 ref. En, de] [Rijksinst. voor de Volksgezondheid, Postbus 1, Bilthoven, Netherlands]

2 outbreaks of *B. cereus* food poisoning in Rotterdam (Netherlands) were investigated. 9 persons were involved, all of whom had consumed dishes containing fried rice about 2 h before developing food poisoning symptoms. The dishes had been obtained from a Chinese restaurant. A bacteriological examination was made of the boiled rice from which the fried rice had been prepared and of the finished dish. Findings prompted a systematic investigation of the bacteriological quality of boiled and fried rice in Chinese restaurants in the Rotterdam area. It was found that slow cooling of the boiled rice, high holding temp. of the boiled rice prior to consumption and the sparing nature of the secondary heat treatment (i.e. frying) in which only the surfaces layers of the rice mass were heated all contributed to high bacterial counts. Under such circumstances the

presence of *B. cereus* in the uncooked rice and the relatively high heat resistance of the organism may lead to food poisoning. JA

106

[*Staphylococcus aureus* growth and survival in macaroni dough and the persistence of enterotoxins in the dried products.]

Lee, W. H.; Staples, C. L.; Olson, J. C., Jr.

Tecnica Molitoria 27 (6) 88-91 (1976) [14 ref. It] [FDA, Washington, DC, USA]

See FSTA (1975) 7 6M717.

107

Spontaneous appearance of staphylococcal variants during vacuum concentration of reconstituted skim milk.

Ghodekar, D. R.; Nambudripad, V. K. N.

Indian Journal of Dairy Science 28 (4) 306-308 (1975) [12 ref. En] [Nat. Dairy Res. Inst., Karnal, India]

The morphological, cultural, biochemical and pathogenicity (coagulase test and DNAase test) characteristics of 3 staphylococcal variants of strain S-6 isolated from reconstituted skim-milk subjected to vacuum concn. were determined. Negative results were given by the variants, BGY-S6 and BPW-S6 to the pathogenicity tests; therefore such results should not be taken as indicative of absence of enterotoxigenic staphylococci in dried milks. LMB

108

Incidence of *Salmonella* spp., *Clostridium botulinum*, and *Vibrio parahaemolyticus* in an estuary.

Sayler, G. S.; Nelson, J. D., Jr.; Justice, A.; Colwell, R. R.

Applied and Environmental Microbiology 31 (5) 723-730 (1976) [20 ref. En] [Dep. of Microbiol., Univ. of Maryland, College Park, Maryland 20742, USA]

A study of the incidence of *Salmonella* spp., *V. parahaemolyticus*-like organisms, and *Cl. botulinum* in samples [including shellfish] collected at 5 stations located in the Upper Chesapeake Bay, a major estuary on the Atlantic Coast of the USA, was conducted in Dec. 1973-Dec. 1974. Shellfish examined during the investigation were found to be free of these enteric pathogens. AS

109

[Effect of post-transport fatigue of slaughter pigs on bacterial contamination of carcass and internal organs.]

Nowicki, L.

Medycyna Weterynaryjna 32 (4) 229-231 (1976) [22 ref. Pl, ru, en] [Katedra Higieny Produktów Zwierzecych, Wydział Weterynaryjny, AR, Lublin, Poland]

Bacterial contamination was studied of 120 samples each of (i) muscle tissue (fore and hind

legs), (ii) lymph glands, (iii) kidneys and (iv) spleen from 120 carcasses of pigs slaughtered directly after tiring lorry transport over 50-100 km (DS); and of 75 corresponding samples each from 75 carcasses of pigs similarly transported but slaughtered after rest for 24 h (RS). Mean numbers/g tissue with ranges of saprophytic microorganisms were for DS and RS respectively: (i) 8.3×10^4 (5.0×10^2 - 1.1×10^6) and 4.9×10^3 (2.0×10^2 - 3.1×10^4), (ii) 1.4×10^5 (1.0×10^3 - 2.9×10^6) and 1.8×10^4 (5.0×10^2 - 1.5×10^5), (iii) 1.2×10^5 (4.0×10^2 - 2.0×10^6) and 7.9×10^3 (2.0×10^2 - 6.4×10^4), and (iv) 1.1×10^5 (2.0×10^2 - 1.9×10^6) and 8.5×10^3 (4.0×10^2 - 9.8×10^4). Numbers of samples containing clostridia were for DS and RS respectively: (i) 20 and 3, (ii) 40 and 9, (iii) 40 and 11, and (iv) 35 and 8, the differences being significant; but there was no difference in extent of combination of the infected samples (overall range, 5.5×10^2 - 3.5×10^3 /g). pH of (i) was 5.96 (5.4-6.5) in DS and 5.75 (5.3-6.4) in RS. SKK

110

Perfringens illness associated with beef prime rib served in a restaurant. [Letter]

Pace, P. J.; Duncan, C. L.; Dowell, V. R.; Antonmattei, J. C.; Wisniewski, H. J.

Journal of Milk and Food Technology 39 (4) 305 (1976) [1 ref. En] [Bureau of Lab., Milwaukee Health Dep., 841 North Broadway, Milwaukee, Wisconsin 53202, USA]

This food poisoning outbreak, which occurred in Milwaukee, Wisconsin in March 1975, involved 28 persons who had consumed prime ribs of beef in one restaurant approx. 6-17 h before developing symptoms (diarrhoea, cramp, nausea, vomiting). Symptoms persisted for 1-30 h. A portion of the beef and stools of 3 patients were examined microbiologically. Clostridium perfringens was isolated from beef and stools; isolates were identified as toxigenic type A. The restaurant stated that ribs are supplied in sealed cryovac wrapping, processed in a commercial rib oven for >3 h then held at 150-160°F for 6-10 h. On the day of the outbreak one rib oven was malfunctioning and ribs were reprocessed in a conventional oven. JA

111

Stability of staphylococcal enterotoxin A to selected conditions encountered in foods.

Chordash, R. A.

Dissertation Abstracts International, B 36 (9) 4307-4308: Order No. 76-5914 (1976) [En] [Cornell Univ., Ithaca, New York 14850, USA]

Stability of the enterotoxin to interactions with reducing sugars and antimicrobial food additives, the effects of microbial growth, and enterotoxin reactivation after partial thermal inactivation were studied, the biological activity of the enterotoxin being measured by immunodiffusion. Results with xylose, lactose, glucose, maltose and fructose indicated that complexing, if it occurred at all, did not reduce enterotoxin titer relative to controls for

any sugar under any of the conditions examined. All sugars had a protective effect on enterotoxin when samples were heated at 60°C. Of the food additives, sodium propionate and sodium benzoate at 0.01, 0.10 and 1.0% levels and NaNO₂ at 5, 50 and 500 ppm did not alter enterotoxin titer on incubation for ≤9 days at 21° or 37°C. The same result was obtained with 10, 100 and 1000 ppm Na₂SO₃ after incubation at 37°C for 3, 6 and 9 days; but, samples containing 1000 ppm Na₂SO₃ incubated for ≤9 days at 21°C or for ≤1 day at 37°C revealed non-detectable enterotoxin titers. Growth of proteolytic bacteria (e.g. Bacillus), enteric bacteria (e.g. Escherichia) and Candida and Saccharomyces yeasts generally had little or no effect on enterotoxin titers. Significant reductions in titers were achieved under some conditions with lactic acid bacteria. Partial enterotoxin reactivation took place with samples heated (or unheated) in 0.02M phosphate-buffered saline when subsequent incubation was at 30° or 37°C for 72 h. JA

112

[Staphylococcal food poisoning in a children's home.] Staphylogene Lebensmittelvergiftung in einem Kinderheim.

Grau, A.

Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, IB 162 (1/2) 211-216 (1976) [1 ref. De, en] [Hygiene Inst. der Ruhrgebiets, Gelsenkirchen, Federal Republic of Germany]

An outbreak of food poisoning involving 56 children at a convalescent home is described. The outbreak was attributable to porridge contaminated with staphylococci; the porridge oats had been soaked at 25°C overnight. 56 of 58 children who had eaten the porridge were affected, whereas none of 16 children who had not eaten the porridge suffered food poisoning. Pathogenic staphylococci were isolated from stomach contents and vomit from the patients; the phage types of these isolates closely with those of isolates from the throat, nose and hands of personnel involved in preparation of the porridge. AJDW

113

[An outbreak of food poisoning caused by Clostridium perfringens.]

Sugitani, A.; Ohashi, T.; Tokoro, M.; Kitano, K. *Journal of the Food Hygienic Society of Japan [Shokuhin Eiseigaku Zasshi]* 16 (6) 424-430 (1975) [18 ref. Ja, en] [Gifu Prefecture Inst. of Public Health, Nagamori, Gifu-shi, Japan]

An outbreak of food poisoning in Gifu Prefecture in Sept. 1970 involving 494 people was considered to be caused by heat-resistant Cl. perfringens which had contaminated Chinese cabbage in lunches consumed by those affected.

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